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Tensegrity I. Cell structure and hierarchical systems biology

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"...The fact that the germ-cell develops into a very complex structure is no absolute proof that the cell itself is structurally a very complicated mechanism: nor yet does it prove, though this is somewhat less obvious, that the forces at work or latent within it are especially numerous and complex..."
D'Arcy W. Thompson (*Growth and Form*, 1917)

Summary

In 1993, a Commentary in this journal described how a simple mechanical model of cell structure based on tensegrity architecture can help to explain how cell shape, movement and cytoskeletal mechanics are controlled, as well as how cells sense and respond to mechanical forces (*J. Cell Sci.* 104, 613-627). The cellular tensegrity model can now be revisited and placed in context of new advances in our understanding of cell structure, biological networks and mechanoregulation that have been made over the past decade. Recent work provides strong evidence to support the use of tensegrity by cells, and mathematical formulations of the model predict many aspects of cell behavior. In addition, development of the tensegrity theory and its translation into mathematical terms are beginning to allow us to define the relationship between mechanics and biochemistry at the molecular level and to attack the larger problem of biological complexity. Part I of this two-part article covers the evidence for cellular tensegrity at the molecular level and describes how this building system may provide a structural basis for the hierarchical organization of living systems — from molecule to organism. Part II, which focuses on how these structural networks influence information processing networks, appears in the next issue.

Key words: Cytoskeleton, Microfilaments, Microtubules, Intermediate filaments, Integrins, Cell shape, Cell mechanics

Introduction

Cellular biochemistry plays out in a world of structural complexity that is nothing like the controlled solution of a test tube. Rather than being filled with a liquid `protoplasm' as imagined a century ago, eukaryotic cells contain an intricate molecular framework, the cytoskeleton, composed of interconnected microfilaments, microtubules and intermediate filaments within their viscous cytosol (Heuser and Kirschner, 1980; Fey et al., 1984). Cytoskeletal filaments both generate and resist mechanical loads, and they are largely responsible for the cell's ability to resist shape distortion. These scaffolds also function as tracks for the movement of organelles, and they orient many of the enzymes and substrates involved in biochemical reactions that mediate critical cellular functions (Ingber, 1993a; Janmey, 1998). Moreover, cells respond to mechanical forces and to changes in cell shape or cytoskeletal structure by altering these same chemical activities (reviewed in Chicurel et al., 1998).

So how do the distinct molecular components of the cytoskeleton contribute to cell mechanics, cell shape control and cellular mechanochemistry? Unfortunately, although great advances have been made in our understanding of the polymerization behavior and physical properties of isolated cytoskeletal filaments and gels, material properties measured in vitro cannot predict mechanical behaviors observed in living cells (Janmey et al., 1991; Gittes et al., 1993). Those biologists who do study mechanical behavior at the whole cell level generally focus on the load-bearing function of the cortical (submembranous) cytoskeleton and ignore the internal cytoskeletal lattice (Albrecht-Buehler, 1987). Mechanical models of the cell similarly depict the cell as an elastic membrane or cortex surrounding a homogeneous cytoplasm that is viscous, viscoelastic or elastic, sometimes with a nucleus in its center (Evans and Yeung, 1989; Dong et al., 1991; Fung and Liu, 1993; Schmid-Schönbein et al., 1995). This view of the cell as a `tensed balloon filled with molasses or jello', however, is of little use when one tries to understand how mechanical forces regulate cell behavior, because it ignores internal microstructure. We must therefore search for a model of the cell that will allow us to relate mechanics to chemistry at the molecular level and to translate this description of the cell into mathematical terms. The former will permit us to define how specific molecular components contribute to complex cell behaviors. The latter will allow us to develop computational approaches to address levels of complexity and multi-component interactions that exist in living cells but cannot be described by current approaches. The long-term goal is to understand biological processes responsible for cell behavior as integrated, hierarchical systems rather than as isolated parts.

In this two-part Commentary, I discuss a model of the cell based on `tensegrity architecture' that appears to meet these goals (Ingber et al., 1981; Ingber and Jamieson, 1982; Ingber and Jamieson, 1985; Ingber, 1993b). Here, in Part I, I examine the evidence that the cytoskeleton that mechanically stabilizes the cell is a tensed tensegrity framework composed of molecular struts, ropes and cables on the nanometer scale and examine the utility of computational models based on this theory. I also explore the implications of this theory for how molecules function as elements within more complex

hierarchical structures composed of systems within systems within systems (i.e. cells, tissues and organs). In Part II, which appears in the next issue of JCS (Ingber, 2003), I discuss the implications of the cellular tensegrity model and biocomplexity for our understanding of mechanobiology and biological pattern formation, with a particular focus on how cells harness complex molecular networks, such as gene and protein networks, for information processing.

Cellular tensegrity

Tensegrity is a building principle that was first described by the architect R. Buckminster Fuller (Fuller, 1961) and first visualized by the sculptor Kenneth Snelson (Snelson, 1996). 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). This is clearly seen in the Snelson sculptures, which are composed of isolated stainless steel bars that are held in position and suspended in space by high tension cables (Fig. 1A). The striking simplicity of these sculptures has led to a description of tensegrity architecture as a tensed network of structural members that resists shape distortion and self-stabilizes by incorporating other support elements that resist compression. These sculptures and similar structures composed of wood struts and elastic strings (Fig. 1B) beautifully illustrate the underlying force balance, which is based on local compression and continuous tension (Fig. 2A) that is responsible for their stability. However, rigid elements are not required, because similar structures can be constructed from flexible springs that simply differ in their elasticity (Fig. 1C).

Fig. 1. Tensegrity structures. (A) Triple crown, a tensegrity sculpture, by the artist Kenneth Snelson, that is composed of stainless steel bars and tension cables. Note that this structure is composed of multiple tensegrity modules that are interconnected by similar rules. (B) A tensegrity sphere composed of six wood struts and 24 white elastic strings, which mimics how a cell changes shape when it adheres to a substrate (Ingber, 1993b). (C) The same tensegrity configuration as in B constructed entirely from springs with different elasticities.

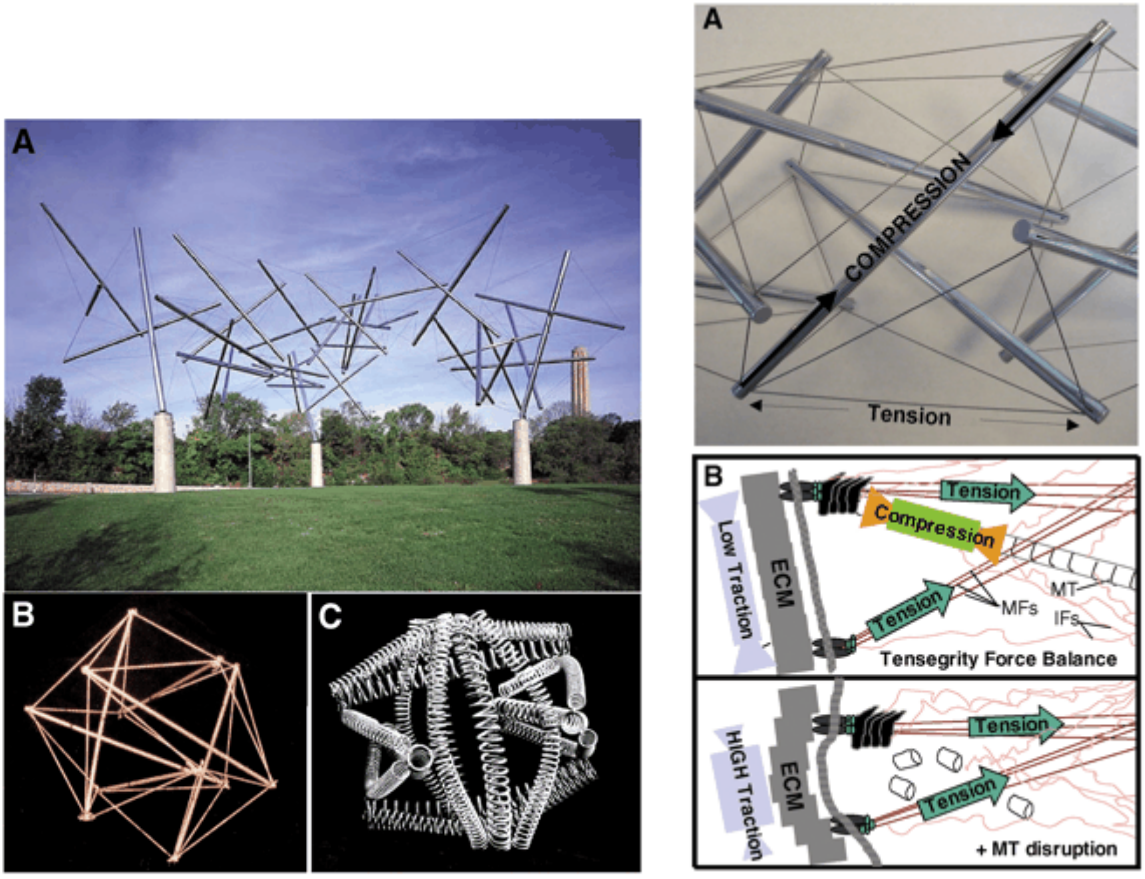

Fig. 2. (A) A high magnification view of a Snelson sculpture with sample compression and tension elements labeled to visualize the tensegrity force balance based on local compression and continuous tension. (B) A schematic diagram of the complementary force balance between tensed microfilaments (MFs), intermediate filaments (IFs), compressed microtubules (MTs) and the ECM in a region of a cellular tensegrity array. Compressive forces borne by microtubules (top) are transferred to ECM adhesions when microtubules are disrupted (bottom), thereby increasing substrate traction.

According to Fuller's more general definition, 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 (Fuller, 1961; Fuller, 1979; Ingber, 1998; Chen and Ingber, 1999). The former hold their joints in position as the result of a `prestress' (pre-existing tensile stress or isometric tension) within the structure (Fig. 1). 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 (Bucky Balls), and tetrahedral space frames, which are popular with NASA because they maintain their stability in the absence of gravity and, hence, without continuous compression.

Some investigators use tensegrity to refer only to the prestressed `bar and cable' structures or particular subclasses of these (e.g. unanchored forms) (Snelson, 1996; Heidemann et al., 2000). Since Fuller defined the term tensegrity, I use his more general definition here. The existence of a common structural basis for these two different classes of structure is also supported by recent work by the mathematician Robert Connelly. He developed a highly simplified method to describe prestressed tensegrity configurations and then discovered that the same fundamental mathematical rules describe the closest packing of spheres (Connelly and Back, 1998), which also delineate the different geodesic forms (Fuller, 1965).

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. 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 extracellular matrix (ECM) adhesions (Fig. 2B). 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. rigid actin filament bundles 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 through their material properties and their ability to act as suspensory cables that interconnect and tensionally stiffen the entire cytoskeleton and nuclear lattice. In addition, the internal cytoskeleton interconnects at the cell periphery with a highly elastic, cortical cytoskeletal 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.

Do cells use tensegrity architecture?

Ten years ago, much circumstantial evidence already supported the idea that cells are prestressed tensegrity structures with internal molecular struts and cables (Ingber, 1993b). For example, biophysical studies with isolated microfilaments and microtubules revealed that the former are better at resisting tension, whereas the hollow microtubules with their higher second moment of inertia are much more effective at withstanding

compression (Mizushima-Sugano et al., 1983). Because of their increased stiffness (persistence length), microtubules are rigid and straight when in solution and even push out long membrane extensions when enclosed within liposomes (Hotani and Miyamoto, 1990), whereas isolated microfilaments and intermediate filaments are bent or highly entangled, respectively (Janmey et al., 1991; Mackintosh and Janmey, 1995). Yet, microtubules often appear to be curved in living cells (Fig. 3A), whereas microfilaments are almost always linear (Fig. 3B). This is consistent with the engineering rule that tension straightens and compression buckles or bends. Linearization of tangled intermediate filaments also occurs during cell spreading (Fig. 3C) as a result of outward extension of the whole network, which depends on the presence of intact microtubules (Maniotis et al., 1997a); actomyosin-based tension instead promotes inward retraction of the network (Tint et al., 1991). In fact, studies of both cultured cells and whole tissues indicate that cell shape stability depends on a balance between microtubules and opposing contractile microfilaments or intermediate filaments (Burnside, 1971; Tomasek and Hay, 1984; Domnina et al., 1985; Vasiliev, 1987; Madreperla and Adler, 1989; Bailly et al., 1991; Maniotis et al., 1997a; Brown et al., 2001).

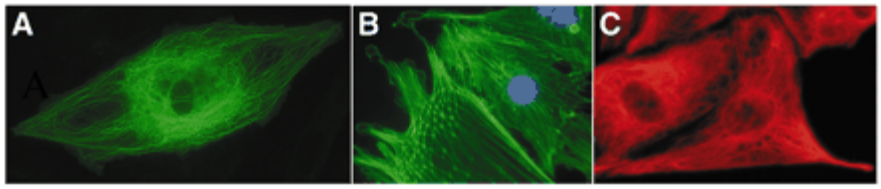

Fig. 3. Microtubules, microfilaments and intermediate filaments within the cytoskeleton of endothelial cells visualized with GFP-tubulin, rhodaminated-phalloidin and antibodies to vimentin, respectively. (A) Microtubules (green) span large regions of the cytoplasm and often appear curved in form. (B) Microfilaments (green-yellow) appear linear in form within long stress fibers and triangulated actin `geodomes'; blue staining indicates nuclei. (C) Intermediate filaments (red) appear within a spread cell as a reticulated network that extends from the nucleus to the cell periphery.

Given these observations and the finding that cells exert tensional forces on their ECM adhesive substrate (Harris et al., 1980), some investigators were initially receptive to the tensegrity model; however, others remained sceptical (Brookes, 1999). Following arguments for and against the model (Heidemann et al., 2000; Ingber, 2000a), it has become clear that experimental validation of the cellular tensegrity model requires convincing demonstration of three major behaviors of living cells. First, cells must behave mechanically as discrete networks composed of different interconnected cytoskeletal filaments and not as a mechanical (e.g. viscous or viscoelastic) continuum. Second, and most critical, cytoskeletal prestress should be a major determinant of cell deformability. And, finally, microtubules should function as compression struts and act in a complementary manner with ECM anchors to resist cytoskeletal tensional forces and,

thereby, establish a tensegrity force balance at the whole cell level. Below, I describe the evidence demonstrating these behaviors that has accumulated over the past decade.

The cytoskeleton behaves like a discrete mechanical network

Established models of cell mechanics developed by biologists and engineers assume that the dense cortical microfilament network that lies directly beneath the cell membrane is the primary load-bearing element in the cell (Albrecht-Buehler, 1987; Evans and Yeung, 1989; Dong et al., 1991; Fung and Liu, 1993; Schmid-Schönbein et al., 1995). These models predict that externally applied stresses are transmitted into the cell equally at all points on the cell surface and are borne exclusively by the cell cortex. In contrast, the tensegrity model predicts that mechanical loads are borne by discrete molecular networks that span the cell surface and extend through the cytoplasm. More specifically, transmembrane molecules that physically couple extracellular anchors (e.g. ECM molecules or cell-cell adhesions) to the internal cytoskeletal lattice should provide preferred paths for mechanical stress transfer into the cell, whereas other transmembrane receptors would dissipate stress locally and thus fail to transmit the same signals. If the cell is a prestressed tensegrity structure, then a local stress can result in global structural rearrangements, even at a distance. This is because the discrete structural elements within the load-bearing network change orientation and spacing relative to one another until a new equilibrium configuration is attained (Fig. 4A). Thus, tensegrity differs from conventional models of the cell in that application of local stresses on the cell surface may result in directed deformation of structures, both locally and deep inside the cell, depending on the molecular connectivity across the surface membrane and through the viscous cytosol.

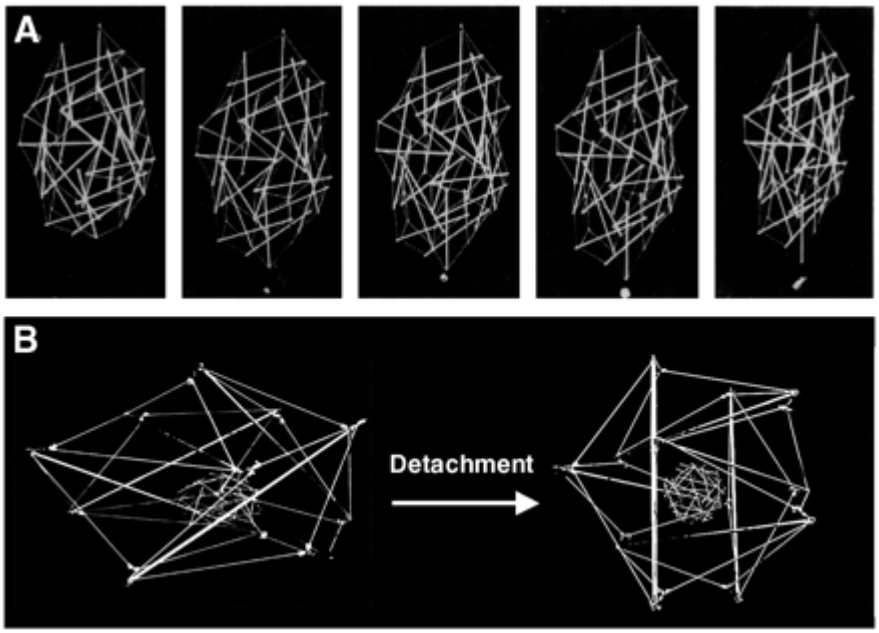

Fig. 4. Tensegrity cell models composed of sticks-and-strings. (A) A model was suspended from above and loaded, from left to right, with 0, 20, 50, 100 or 200 g weights on a single strut at its lower end. Note that a local stress induces global structural arrangements. Reprinted

(abstracted/excerpted) with permission from (Wang et al., 1993) American Association for the Advancement of Science. (B) A tensegrity model of a nucleated cell when adherent and spread on a rigid substrate (left) or detached and round (right). The cell model is composed of large metal struts and elastic cord; the nucleus contains sticks and elastic strings. In this cell model, the large struts conceptually represent microtubules; the elastic cords correspond to microfilaments and intermediate filaments that carry tensional forces in the cytoskeleton.

Ning Wang and I set out to discriminate between these conflicting models by developing a micromanipulation method called magnetic twisting cytometry, in which controlled mechanical stresses are applied directly to cell-surface receptors by applying torque (shear stress) to receptor-bound magnetic microbeads (~1 to 10 μm diameter) (Wang et al., 1993; Wang and Ingber, 1994; Wang and Ingber, 1995). In separate studies, magnetic tweezers (Bausch et al., 1998; Alenghat et al., 2000) were developed and used to apply linear tensional stresses to cells, and optical tweezers were utilized to manipulate non-magnetic beads that were similarly bound to cell-surface receptors (Schmidt et al., 1993; Choquet et al., 1997).

These techniques revealed that cell-surface adhesion receptors, such as integrins, that link to the internal cytoskeleton provide a greater degree of mechanical coupling across the cell surface than do other transmembrane molecules, even though all connect to the submembranous cytoskeleton (i.e. the actin-spectrin-ankyrin lattice). For example, when we used magnetic twisting cytometry to stress transmembrane acetylated-low density lipoprotein (AcLDL) metabolic receptors or histocompatibility antigens, there was detectable, but minimal, resistance to mechanical distortion (Wang et al., 1993; Yoshida et al., 1996). In contrast, when ECM-ligandcoated beads bound to ß1 integrins were similarly stressed, the cells responded by increasing their stiffness in direct proportion to the applied stress. Importantly, we could partially inhibit the integrin-dependent stiffening response by disrupting microfilaments, microtubules or intermediate filaments, and completely prevent it by disrupting all at once (Wang et al., 1993). Thus, although each cytoskeletal filament system imparts mechanical stiffness, the mechanical properties of the cell are not determined by the material properties of any single type of molecular filament. The same finding has been obtained in studies with non-adherent, circulating lymphocytes (Brown et al., 2001). Cellular mechanical behavior is therefore an emergent property that results from collective interactions among all three filament systems.

Differences in transmembrane mechanical coupling depend on the ability of the receptor to form a membrane adhesion complex that physically links to the internal cytoskeletal lattice. For example, binding of magnetic beads to ß1 integrins induces formation of molecular links to the internal cytoskeleton, as indicated by local assembly of focal adhesions containing integrins, associated actin-binding proteins (e.g. vinculin, talin and α-actinin) and filamentous actin at the site of bead binding (Plopper and Ingber, 1993;

Wang et al., 1993). Moreover, cells from mice lacking vinculin exhibit a large drop in transmembrane mechanical coupling that is independent of integrin binding and can be restored by transfection of the cells with this focal adhesion protein (Ezzell et al., 1997; Alenghat et al., 2000). In optical tweezer studies, beads bound to cell-surface integrins also exhibit very little resistance to stress during the first seconds to minutes after binding; however, once the integrins have formed focal adhesions, the beads stiffen so that they can no longer be displaced (Schmidt et al., 1993; Choquet et al., 1997). Local recruitment of focal adhesion proteins to integrin-binding sites also can be induced by pulling on integrins with ECM-coated micropipettes in conjunction with a micromanipulator (Riveline et al., 2001). This effect is mediated by an increase in cytoskeletal tension, either activated internally by the GTPase Rho and its downstream target Rho-associated kinase (ROCK) or by external application of tension to the cytoskeleton via integrins in the presence of the active form of another downstream Rho target, mDia1.

When larger mechanical stresses are applied to transmembrane integrin receptors on living cells, using ligand-coated micropipettes, both local and distant effects are observed. Application of these higher forces to integrins and associated focal adhesions results in physical distortion of the surface membrane and immediate repositioning of cytoskeletal filaments along the applied tension field lines within the cytoplasm (Fig. 5A,B), as well as realignment of molecular elements within nucleoli deep in the center of the nucleus (Fig. 5C-F) (Maniotis et al., 1997a). Application of tension to transmembrane AcLDL receptors produces no such changes. Cells that lack intermediate filaments fail to support efficient mechanical coupling between integrins and the nucleus; instead tension produces cytoplasmic tearing (Maniotis et al., 1997a; Eckes et al., 1998). Intermediate filament disruption also destabilizes the microtubule and microfilament networks (Goldman et al., 1996). Yet, the intermediate filament lattice alone is sufficient to provide some mechanical stiffness to the cell as shown, for example, when lymphocytes that are devoid of intact microfilaments or microtubules are compressed against a substrate by centrifugation (Brown et al., 2001).

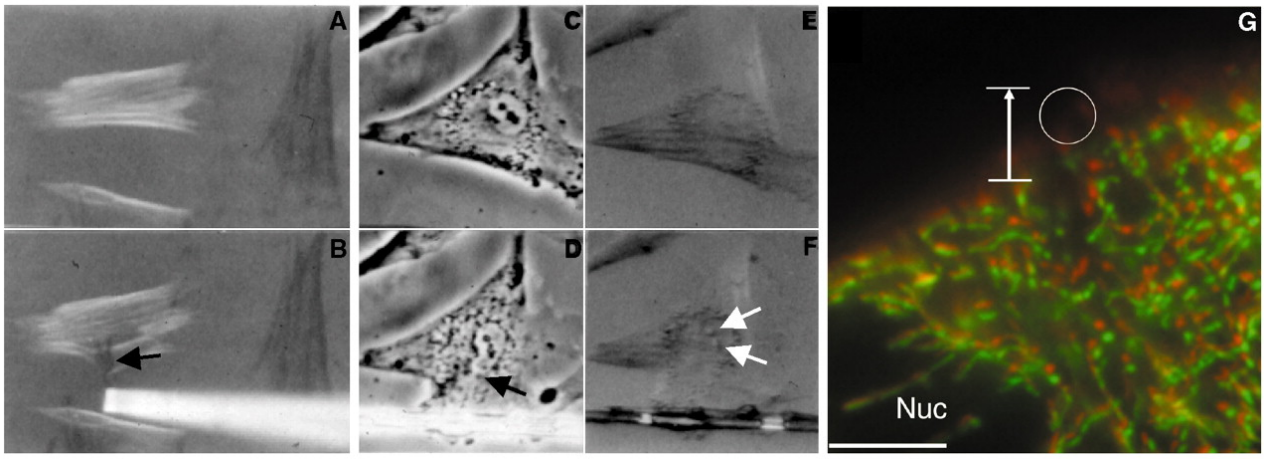

Fig. 5. Force transfer through discrete molecular networks in living cells. Polarization optics (A,B,E,F), phase contrast (C,D) and fluorescence (G) views of cells whose integrin receptors were mechanically stressed using surface-bound glass micropipettes coated with fibronectin (A-F) or uncoated micropipettes with ECM-coated microbeads (G). (A) Cells exhibiting positively (white) and negatively (black) birefringent cytoskeletal bundles aligned horizontally and vertically, respectively, in the cytoplasm of adherent cells. (B) Birefringent cytoskeletal bundles that originally appeared white in A immediately changed to black (black arrow) as they turned 90° and realigned vertically along the axis of the applied tension field when integrins were pulled laterally (downward in this view). (C,E) An adherent cell immediately before a fibronectincoated micropipette was bound to integrin receptors on its surface and pulled laterally (downward in this view) using a micromanipulator as shown in D,F. (D) The black arrow indicates nuclear elongation and downward extension of the nuclear border along the applied tension field lines. (F) White arrows abut on white birefringent spots that indicate induction of molecular realignment within nucleoli in the center of the nucleus by applying mechanical stress to integrins microns away on the cell surface (see Maniotis et al., 1997a). (G) A cell containing EYFP-labeled mitochondria that was stressed by pulling on a surface-bound RGD-microbead using a micromanipulator. Vertical arrow, direction and extent of bead displacement; white circle, position of bead after stress application; green, position of mitochondria before stress application; red, their position approximately 3 seconds after stress was applied; Nuc, nucleus of the cell. Note that long distance transfer of mechanical force across integrins result in movement of mitochondria deep in the cytoplasm. Panel G reproduced with permission from the National Academy of Sciences (Wang et al., 2001).

Other studies used micropipettes to pull on microbeads bound to integrins on living cells transfected with a construct that produces a fusion protein of enhanced yellow fluorescent protein (EYFP) and cytochrome C oxidase to make mitochondria fluorescent. Real-time fluorescence microscopic analysis revealed coordinated movement of mitochondria as far as 20 μm into the cell (Fig. 5G) (Wang et al., 2001). Again, pulling on transmembrane AcLDL receptors that couple only to the membrane cortex failed to produce this effect. Mitochondria directly associate with microtubules and are excluded from the cell cortex. Thus, forces transmitted by integrins to microfilaments in the focal adhesion apparently can be passed to microtubules at distant sites and so these different filament networks must be mechanically connected inside living cells. Application of fluid shear stresses to the apical cell surface of cultured endothelium also results in mechanical distortion of GFP-labeled intermediate filaments deep inside the cytoplasm (Helmke et al., 2001).

Thus, the cellular response to stress does depend on connectivity within discrete molecular networks that span the cell surface and extend through the cytoplasm, and on cooperative interactions between all three cytoskeletal filament systems. The data discussed above therefore provide direct support for the tensegrity model and are not consistent with models that view the cell as an elastic membrane surrounding a viscous cytosol. These studies, however, also reveal a caveat. Even though the internal

cytoskeletal lattice is clearly critical for the cellular response to mechanical stress, the cell may appear to behave like an elastic cortex surrounding a viscous cytosol, if the highly elastic, submembranous cytoskeletal network is probed independently of the internal cytoskeletal lattice. This was observed in experiments in which non-adhesion receptors (Wang et al., 1993; Wang and Ingber, 1994; Wang and Ingber, 1995) or inactive (unligated) integrins (B. Mathews, F. Alenghat and D.E.I., unpublished) were magnetically twisted, and when activated integrins were pulled in the plane of the membrane (Bausch et al., 1998). This caveat might also explain why only local responses are observed when mechanical stresses are applied to cell surfaces by micropipettes coated with laminin (Heidemann et al., 1999); in this study, efficient mechanical coupling between cell surface adhesion receptors and the internal cytoskeleton (i.e. focal adhesion formation) does not appear to occur.

Prestress is a major determinant of cell mechanics

The most fundamental feature of the cellular tensegrity model is the importance of tensional integrity and internal tensile stress (prestress) for cell shape stability. There is no question that mammalian cells experience isometric tension, because this can be visualized if one plates cells on flexible substrates (Harris et al., 1981) or quantifies cell-generated forces (Kolodney and Wylomerski, 1992; Pelham and Wang, 1997; Wang et al., 2001; Balaban et al., 2001). Microsurgical techniques can also demonstrate this directly: sever the cell anywhere and the cut edges spontaneously retract (Pourati et al., 1998). Engineers use a similar technique to quantify prestress (residual stress) within whole living tissues and organs (Fung and Liu, 1989; Omens and Fung, 1990). Altering cytoskeletal prestress by modulating actomyosin-based contractility using drugs (Hubmayr et al., 1996; Wang et al., 2001), varying transmembrane osmotic forces (Cai et al., 1998), transfecting cells with constitutively active myosin light chain (MLC) kinase (Cai et al., 1998) or quickly distending a cell's adhesive substrate (Pourati et al., 1998) also results in immediate changes in cell shape stability (shear modulus). Most importantly, experimental measurement of cultured cells, using traction force microscopy to quantify prestress within individual cells (Pelham and Wang, 1997; Butler et al., 2002) and magnetic twisting cytometry to measure cell stiffness, reveals a linear correlation between stiffness (elastic modulus) and cellular prestress (Wang et al., 2002), as predicted a priori by the tensegrity model (Stamenovic et al., 1996). Cells also exhibit a nearly linear dependence of their dynamic mechanical behavior (dynamic modulus) on cytoskeletal prestress (Stamenovic et al., 2002a).

Those who view cell mechanics as largely a function of the elastic cell cortex might ascribe these results to the importance of tensional prestress in the cortical cytoskeleton. However, measurements of cell mechanics using magnetic twisting cytometry in conjunction with two different-sized magnetic beads conflict with this interpretation; cell stiffness scales directly with bead size for a given applied stress, which is the opposite of what would be predicted by a prestressed membrane cortex model (Wang and Ingber, 1994). Moreover, no change in mechanics can be detected in round versus flat cells or in cells expressing constitutively active MLC kinase when they are probed with techniques that measure only the cortical cytoskeleton (Wang and Ingber, 1994; Cai et al., 1998). In contrast, major differences are evident in the same cells when one measures cell

mechanics through integrins that couple to the internal cytoskeleton by magnetic twisting cytometry. Differences in shape stability owing to altered prestress therefore cannot be explained solely on the basis of changes in the cell cortex.

Cytoskeletal prestress is also important for shape stability in the cytoplasm and nucleus. For example, addition of ATP to membrane-permeabilized cells results in coordinated retraction and rounding of the entire cell, cytoskeleton and nucleus, and this response can be prevented by blocking cytoskeletal tension generation (Sims et al., 1992). Tensegrity models of nucleated cells composed of struts and tensed cables (Fig. 4B) exhibit similar coordinated retraction behavior when their anchors are dislodged. Moreover, quantification of changes in cell stiffness in membrane-permeabilized cells using magnetic twisting cytometry confirmed that cytoskeletal tension (prestress) is a critical determinant of cell and nuclear shape stability independently of transmembrane osmotic forces (Wang and Ingber, 1994). The stiffness of the cell, cytoskeleton and nucleus also can be altered by disruption of the tensed intermediate filament lattice by drugs (Wang et al., 1993; Maniotis et al., 1997a; Wang and Stamenovic, 2000; Brown et al., 2001), synthetic inhibitory peptides (Goldman et al., 1996) or genetic techniques [e.g. vimentin-knockout mice (Eckes et al., 1998; Wang and Stamenovic, 2000; Brown et al., 2001)] or by modifying the ability of the ECM substrate to resist cell traction (Wang and Ingber, 1994). Thus, as predicted by the tensegrity model, continuous transmission of tension between different cytoskeletal filament systems, and from the cytoskeleton to both the nucleus and ECM receptors, is critical for cell shape stability. Interestingly, even the submembranous cytoskeleton (the cortical actin-ankyrin-spectrin lattice) appears to require tensional prestress for its mechanical stability (Discher et al., 1998; Coughlin and Stamenovic, 2003).

Establishment of a tensegrity force balance between microtubules, microfilaments and ECM

The feature of the cellular tensegrity model that most troubles investigators is the presence of compression struts inside the cell. Some argue that the cytoskeleton is like a network of muscles, tendons and ligaments without the bones (Brookes, 1999). So where are the compression elements? The answer depends on the size scale and hierarchical level that one examines. From the physiological perspective, the most relevant level relates to how the cell controls its shape and structure within living tissues. When cells are enzymatically dislodged from tissues, they spontaneously round up and lose their characteristic forms. When the ECM is carefully removed from developing tissues without disrupting cell-cell contacts, cells do not completely round up; however, they partially retract and lose specialized tissue morphology, such as epithelial branches and buds (Banerjee et al., 1977). In other words, cells cannot stabilize their specialized shapes in the absence of their ECM adhesions. Thus, one cannot define the critical determinants of cell shape stability in anchorage-dependent cells without considering the mechanics of the adhesion substrate, just as one cannot describe the stability of a spider web without considering the tree branches to which it is tethered.

Studies of cultured cells confirm that cell shape depends on the ability of local regions of the ECM anchoring substrate to withstand compression. Cells are not evenly glued to

their adhesive substrate, rather they are spot welded in regions known as focal adhesions (Burridge et al., 1988) that contain clustered integrin receptors and cytoskeleton-coupling proteins as well as immobilized signal transduction molecules (Plopper and Ingber, 1993; Plopper et al., 1995; Miyamoto et al., 1995). Focal adhesions generally form at the base of the cell directly beneath the ends of each contractile stress fiber (Burridge et al., 1988); thus, they represent discrete points of cytoskeletal insertion on the ECM analogous to muscle-insertion sites on bone. To support cell spreading, isolated regions of the extracellular substrate located between focal adhesions must resist local compression produced by the shortening of each internal stress fiber. It is for this reason that adherent cells pull flexible substrates up into `compression wrinkles' between their localized adhesions (Harris et al., 1980). Thus, these local regions of the ECM act like external support elements to resist cytoskeletal tensional forces and thereby establish a tensegrity force balance.

If these ECM regions were the only elements that resisted cell tension, then all cells adherent to planar ECMs would look like fried eggs. This is not the case, because cells also use internal compression struts to refine their shape. During neurulation in the embryo, developing epithelial cells extend internal microtubule struts along their vertical (apical-basal) axis to transform themselves into columnar cells (Burnside, 1971). One can also induce round lymphocytes (Bailly et al., 1991) and erythrocytes (Winckler and Solomon, 1991) to form long membrane extensions by promoting microtubule polymerization. If microtubules did not resist compression and were tensed like rubber bands, then these cells would not be able to create highly elongated forms, and spherical contraction would result. In other words, these cells must contain some internal element that resists inward-directed cytoskeletal forces in order to extend outward; this is a key feature of tensegrity architecture.

The remaining concern that has been raised is whether long microtubules that extend throughout the cytoplasm of cultured cells actually bear compression. To envision how this might work in the tensegrity model more clearly, think of a camp tent. The surface membrane of the tent is stabilized (made stiff) by placing it under tension. This can be accomplished by various means: pushing up tent poles against the membrane, pulling the membrane against fixed tent pegs in the ground and tethering the membrane to an overlying tree branch. The internal tent poles and external tethers provide complementary load-bearing functions because both resist the inward-directed forces exerted by the tent membrane. It is through this tensegrity force balance that the tensional prestress is generated that stabilizes the tent's form.

If cells use tensegrity and the cytoskeleton is organized liked a tent, then if you were to disrupt the microtubules (tent poles), the force they normally carried would be transferred to the cell's adhesive anchors. This transfer of forces would cause increased traction on the cell's adhesions (i.e. the tent pegs would be pulled upward and closer together, and the tree branch would be wrenched downward) (Fig. 2B). By contrast, if all CSK filaments experience tension, like a bunch of tensed rubberbands, then if you were to break any of the filaments, tension on the substrate would rapidly dissipate (the tree branch would leap back up to its starting position). Importantly, many experiments have shown that when

microfilaments or intermediate filaments — the tension elements in the model — are chemically disrupted, cell tractional forces exerted on ECM adhesions decrease (Kolodney and Wyslomerski, 1992; Eckes et al., 1998). Moreover, when microtubules — the struts in the model — are disrupted, traction on the ECM substrate rapidly increases in many cell types and experimental systems (Danowski, 1989; Kolodney and Wyslomerski, 1992; Kolodney and Elson, 1995; Wang et al., 2001; Stamenovic et al., 2002b).

Although these results directly support the tensegrity model, there is one potential concern: microtubule depolymerization also activates MLC kinase (Kolodney and Elson, 1995). This could mean that the observed increase in ECM traction is entirely controlled through a chemical mechanism (e.g. through tubulin monomer release) and a subsequent increase in active tension generation, rather than mechanically through a tensegrity force balance (Danowski, 1989; Kolodney and Elson, 1995). Other investigators have proposed that microtubule-dependent changes in intracellular calcium levels are responsible for these effects (Paul et al., 2000). Importantly, recent studies have shown that microtubule disruption results in an increase in tractional forces exerted on the ECM substrate, even under conditions in which MLC phosphorylation and intracellular calcium levels do not change (Wang et al., 2002; Stamenovic et al., 2002b). Quantification of cell tractional forces and the amount of prestress within individual cells using traction force microscopy revealed that microtubules counterbalance ~5-30% of the total cellular prestress, depending on the cell. Thus, the ability of microtubules to bear compression locally contributes significantly to cellular prestress and cell shape stability. Note that both application of mechanical force to cell-ECM adhesions (Riveline et al., 2001) and microtubule disruption (Liu et al., 1987) activate the Rho signaling pathway that leads to MLC phosphorylation. So tensegrity-based transfer of mechanical loads to ECM adhesion sites following microtubule disruption could, in part, increase active contraction through a mechanochemical mechanism [see Part II of this Commentary for more discussion of tensegrity and mechanochemistry (Ingber, 2003)].

Because of complementary tensegrity-based force interactions between microtubules, contractile microfilaments and ECM adhesions, the relative contribution of microtubules to cellular prestress will vary depending on the structural context. For example, the poles in the tent bear less compressive load when the tent membrane is partially secured to the overlying tree branch. Similarly, microtubules may bear less compression (and the ECM more) in highly spread cells on rigid substrates, whereas more compression will be transferred from the ECM onto these internal struts when the ECM is compliant or when the cell's ECM adhesions are dislodged. Experiments analyzing the effects of ECM adhesion and mechanical forces on microtubule polymerization in various adherent cells (Joshi et al., 1985; Dennerll et al., 1988; Dennerll et al., 1989; Lamoureux et al., 1990; Mooney et al., 1994; Putnam et al., 1998; Putnam et al., 2001; Kaverina et al., 2002) and a thermodynamic model of microtubule regulation (Buxbaum and Heidemann, 1988) support this notion. This may explain why microtubules did not appear to contribute significantly to smooth muscle cell mechanics in a study in which these cells were held under external tension (Obara et al., 2000), whereas in other studies they were found to play an important mechanical role in both smooth muscle cells

(Wang et al., 2001; Stamenovic et al., 2002) and cardiac muscle cells (Tagawa et al., 1997).

It remains difficult for some to envision how a single molecular filament, such as a microtubule, could withstand compressive forces. The ability of individual microtubules to resist buckling when compressed could be greatly enhanced, however, by the presence of lateral tensile connections that would function as molecular guy wires. On the basis of the frequency of lateral connections along microtubules, engineers have calculated that intermediate filaments could provide this function (Brodland and Gordon, 1990). However, electron microscopy reveals many types of lateral molecular linkage that could act in this manner (Heuser and Kirschner, 1980; Fey et al., 1984).

Importantly, microscopic visualization of the dynamics of green fluorescent protein (GFP)-labeled microtubules provides direct evidence of end-on compressive buckling of individual microtubules in living cells (Fig. 6). Buckled microtubules also immediately straighten when they slip by an obstacle in the cytoplasm (Kaech et al., 1996; Wang et al., 2001). Furthermore, the curvature of individual microtubules (a readout of compressive buckling) decreases when drugs are used to decrease cytoskeletal tension, whereas buckling increases when agents are added that increase contraction, such as thrombin in endothelial cells (Waterman-Storer and Salmon, 1997; Wang et al., 2001). Disruption of microtubules also significantly reduces the stiffness (shear modulus) of the cell (Wang et al., 1993; Stamenovic et al., 2002) and induces retraction of long processes in various cell types (Tomasek and Hay, 1984; Domnina et al., 1985; Vasiliev, 1987; Madreperla and Adler, 1989; Bailly et al., 1991; Ingber et al., 1995).

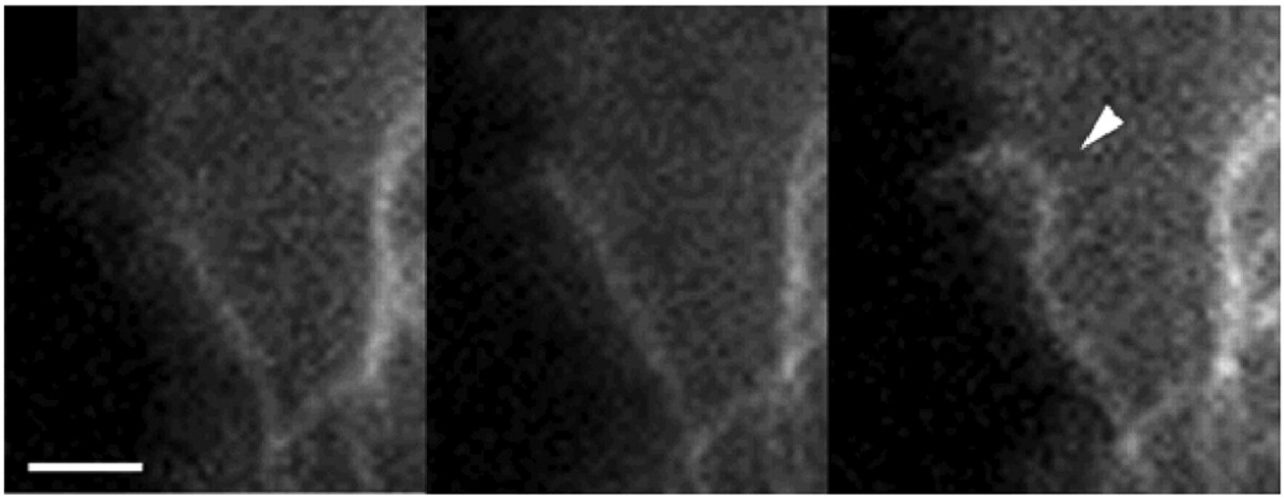

Fig. 6. Three sequential fluorescent images from a time-lapse recording of the same cell expressing GFP-tubulin showing buckling of a microtubule (arrowhead) as it polymerizes (from left to right) and impinges end-on on the cell cortex at the top of the view [reproduced with permission from the National Academy of Sciences (Wang et al., 2001)].

Taken together, these studies indicate that at least a subset of microtubules function as compression struts within the cytoplasm and act in a complementary manner with ECM adhesions to resist microfilament-based tensional forces in the cytoskeleton of adherent cells. In this manner, a tensegrity force balance is established. Moreover, microtubules appear to provide a similar compression-bearing function in the mitotic spindle: Pickett-Heaps and co-workers severed a single microtubule within the spindle with a UV microbeam, and the remaining microtubules buckled as if the total compressive load was distributed among a decreased number of semiflexible compression struts (Pickett-Heaps et al., 1997). However, microtubules have a dual function in that some (kinetochore) microtubules experience tension when they shorten and pull the chromosomes apart and toward the spindle poles during anaphase at the end of mitosis (Zhou et al., 2002).

Mathematical formulation of the tensegrity theory

The cellular tensegrity theory was initially an intuitive model, and prestressed tensegrity structures constructed out of sticks and elastic strings were used to visualize the concept (Ingber and Jamieson, 1985; Ingber, 1993b; Wang et al., 1993). Nevertheless, these simple models closely mimicked living cells. For example, the cell and nucleus of a round tensegrity model spread in a coordinated manner, and the nucleus moves to the base (polarizes) when it attaches to a rigid substrate (Fig. 4B), which is just like living cells in culture (Ingber et al., 1986; Ingber, 1990). Also, like cultured cells, the models contract and wrinkle flexible substrates, and they take on a round form when detached (Ingber, 1993b). In addition, the models exhibit the linear stiffening behavior (strain hardening) displayed by cultured cells (Wang et al., 1993) and whole living tissues (McMahon, 1984), apparently because increasing numbers of the struts realign along the applied tension field lines (Fig. 4A). Another model, composed of multiple soda straws tensionally linked by elastic string, kinematically transforms into three-dimensional forms that closely resemble structures observed within actin geodomes and stress fibers of living cells by light (Fig. 3B) and electron microscopy (Osborn et al., 1978), including strut-for-strut and vertex-for-vertex identity on the nanometer scale (Ingber, 1993b).

Although these conceptual models were impressive, further advance in this field required the development of a mathematical formulation of the cellular tensegrity model. A theoretical formulation of the model starting from first mechanistic principles was developed by Dimitrije Stamenovic working with my group (Stamenovic et al., 1996) and by others (Wendling et al., 1999; Wendling et al., 2002; Volokh et al., 2000; Volokh et al., 2002). In this model, actin microfilaments and intermediate filaments carry the prestress that is balanced internally by microtubules and externally by focal adhesions to the ECM substrate. Work on variously shaped models revealed that even the simplest prestressed tensegrity sculpture embodies the key mechanical properties of all members of this tensegrity class. Thus, for simplicity, we used a symmetrical cell model in which the tensed filaments are represented by 24 cables and the microtubules by six struts organized as shown in the structure in Fig. 1B. The cytoskeleton and substrate together were assumed to form a self-equilibrated, stable mechanical system; the prestress carried by the cables was balanced by the compression of the struts.

A microstructural analysis of this model using the principle of virtual work led to two a priori predictions: (1) the stiffness of the model (or cell) will increase as the prestress (*pre-existing* tensile stress) is raised; and (2) at any given prestress, stiffness will increase linearly with increasing stretching force (*applied* stress). The former is consistent with what we know about how muscle tone alters the stiffness of our bodies, and it closely matches data from experiments with living cells (Wang et al., 2002; Stamenovic et al., 2002a; Stamenovic et al., 2003). The latter meshes nicely with the mechanical measurements of stick-and-string tensegrity models, cultured cells and whole living tissues, although it also can be explained by other models (Heidemann et al., 2000). This mathematical approach strongly supported the idea that the architecture (the spatial arrangement of support elements) and prestress (the level of isometric tension) in the cytoskeleton are key to a cell's ability to stabilize its shape.

Largely through the work of Stamenovic and co-workers, this oversimplified micromechanical model continues to be progressively modified and strengthened over time (Coughlin and Stamenovic, 1997; Coughlin and Stamenovic, 1998; Stamenovic and Coughlin, 1999; Stamenovic and Coughlin, 2000; Stamenovic and Ingber, 2002). A more recent formulation of the model includes, for example, semiflexible struts analogous to microtubules, rather than rigid compression struts, and incorporates values for critical features of the individual cytoskeletal filaments (e.g. volume fraction, bending stiffness and cable stiffness) from the literature (Coughlin and Stamenovic, 1997; Stamenovic and Coughlin, 1999). This more refined model is qualitatively and quantitatively superior to that containing rigid struts. Another formulation of the tensegrity model includes intermediate filaments as tension cables that link the cytoskeletal lattice and surface membrane to the cell center (Wang and Stamenovic, 2000). This model generates predictions of mechanical behavior in the absence of intermediate filaments that closely mimic results obtained in studies of living cells in which vimentin has been knocked out genetically or intermediate filaments have been disrupted by pharmacological approaches.

Moreover, all of these tensegrity models yield elastic moduli (stiffness) that are quantitatively similar to those of cultured adherent cells (Stamenovic and Coughlin, 1999; Stamenovic and Coughlin, 2000). Importantly, although models of the cytoskeleton that incorporate only tensile elements (i.e. they lack internal compression struts) can mimic the cell's response to generalized membrane deformation (e.g. owing to poking of a cell with an uncoated micropipette), they cannot explain many other cell mechanical behaviors, especially those that are measured through cell-surface receptors that link to the internal cytoskeleton (Coughlin and Stamenovic, 2003).

Stamenovic has also carried out an energy analysis using quantitative results from traction force microscopy studies of living cells (Stamenovic et al., 2002b). An energy analysis is independent of microstructural geometry and, thus, it circumvents potential limitations of using a specific tensegrity configuration (network architecture) in the theoretical calculations. This analysis revealed that microtubules contribute significantly to the contractile energy budget of the cell and, thus, it provides independent support for the concept that compression-bearing microtubules play an important role in the

determination of mechanical behavior within adherent cells. In contrast, the amount of contractile energy stored in extension of actin microfilaments was found to be negligible. These results are therefore consistent with the tensegrity model, because they suggest that the primary mechanical role of microfilaments is to carry prestress and to transfer tensional forces throughout the cell, whereas microtubules carry compression and balance a substantial fraction of the contractile prestress within the actin network. Stamenovic's analysis also provided evidence for the notion that intermediate filaments provide a lateral mechanical support to microtubules and thus enhance their ability to carry compression without buckling, as predicted previously (Brodland and Gordon, 1990).

Taken together, these results show that, although the current formulation of the tensegrity theory relies on the use of a highly simplified architecture (six struts and 24 cables), it nevertheless effectively predicts many static mechanical behaviors of living mammalian cells. Most critically, the a priori prediction of the tensegrity model that cell stiffness will increase in proportion with the prestress has been confirmed in various experimental studies (Wang et al., 2002; Stamenovic et al., 2002a; Stamenovic et al., 2003). However, what is more surprising is that this model also leads to predictions of dynamic behavior. For example, it predicts that at a given frequency of loading, both the elastic (storage) and frictional (loss) moduli should increase with increasing prestress, whereas the fraction of the frictional energy loss relative to the elastic energy storage should be independent of prestress. Recent experiments again confirm these predictions (Wang et al., 2001; Stamenovic et al., 2002a).

Interestingly, recent work suggests that the dynamic mechanical behavior of mammalian cells depends on generic system properties, as indicated by a spectrum of time constants when the cells are stressed over a wide range of force application frequencies (Goldmann and Ezzell, 1996; Fabry et al., 2001). This work suggests that these dynamic behaviors reflect a non-deterministic property of the cell at some higher system level of molecular interaction. It is not consistent with the notion of a single type of cytoskeletal filament or molecular interaction (e.g. actin crosslinking) being responsible for cell dynamic behavior. It is also not consistent with standard ad hoc models of cell mechanics that assume that the elastic and frictional behaviors of the cell originate from two distinct compartments (the elastic cortex and the viscous cytoplasm). Importantly, computer simulations suggest that dynamic mechanical behaviors exhibited by living cells, including the dependence of both their elastic and frictional moduli on prestress, are natural consequences of their use of tensegrity (Canadas et al., 2002) (C. Sultan, N. Liang, D. Stamenovic and D.E.I., unpublished). In other words, tensegrity could provide a common structural basis for both the elastic and viscous behaviors of living cells.

Other micromechanical models of the cell have been proposed over the past decade; these are based on porous cellular solids (Satcher and Dewey, 1996), filament dynamics [i.e. thermal fluctuations (MacKintosh and Janmey, 1995)] and percolation theory (Forgacs, 1995). As in the tensegrity theory, these models incorporate microstructure and assume that the cytoskeleton is organized as a porous network composed of discrete structural elements. However, these models differ from tensegrity in that they do not take into account contributions from collective interactions among different cytoskeletal filament

systems (or the ECM) and do not explain how highly organized structures [e.g. actin geodomes (Lazarides, 1976)] appear in the cytoskeleton. More importantly, they do not include a role for cytoskeletal prestress in cell shape stability or lead to a priori predictions of complex mechanical behaviors in whole living cells. Thus, although these or other models of the cell may be able to describe particular cell behaviors (Heidemann et al., 2000), they cannot explain many others (Ingber, 2000a). Only the tensegrity theory provides all these features and, thus, it appears to be the most unified and robust model of the cell available at present.

Incorporating structural complexity: multimodularity

Although the simple six-strut tensegrity model of the cell has been very useful, the reality is that the living cell is more complex because it is a `multimodular' tensegrity structure. By multimodularity, I mean that the cell is composed of multiple smaller, self-stabilizing tensegrity modules that are linked by similar rules of tensional integrity (see the structures in Fig. 7 and the sculpture in Fig. 1A). As long as these modules are linked by tensional integrity, then the entire system exhibits mechanical coupling throughout and an intrinsic harmonic coupling between part and whole (Ingber and Jamieson, 1985; Pienta et al., 1991; Pienta and Coffey, 1991a). Destruction of one unit in a multimodular tensegrity, however, results only in a local response; that particular module will collapse without compromising the rest of the structure. This is similar to cutting the Achilles tendon: foot stability is lost, but control of the remainder of the body remains intact. This point is critical because some have ruled out the relevance of tensegrity as a model for living cells on the basis that, if cells used tensegrity, then disruption of one molecular support element would produce total cellular collapse, as in a single tensegrity module (Forgacs, 1995). The fact that individual fragments of cells continue to exhibit specialized behaviors, including movement (Albrecht-Buehler, 1980), after mechanical disruption of the cell confirms that multiple structural modules exist in the cytoplasm, even though they exhibit spatially coordinated behavior in the whole cell. Use of a multimodular tensegrity arrangement provides another important advantage: subsystems or small groups of modules can be repaired and replaced without disruption of higher-order structure. This is critical because the molecules that comprise living cells undergo continuous turnover.

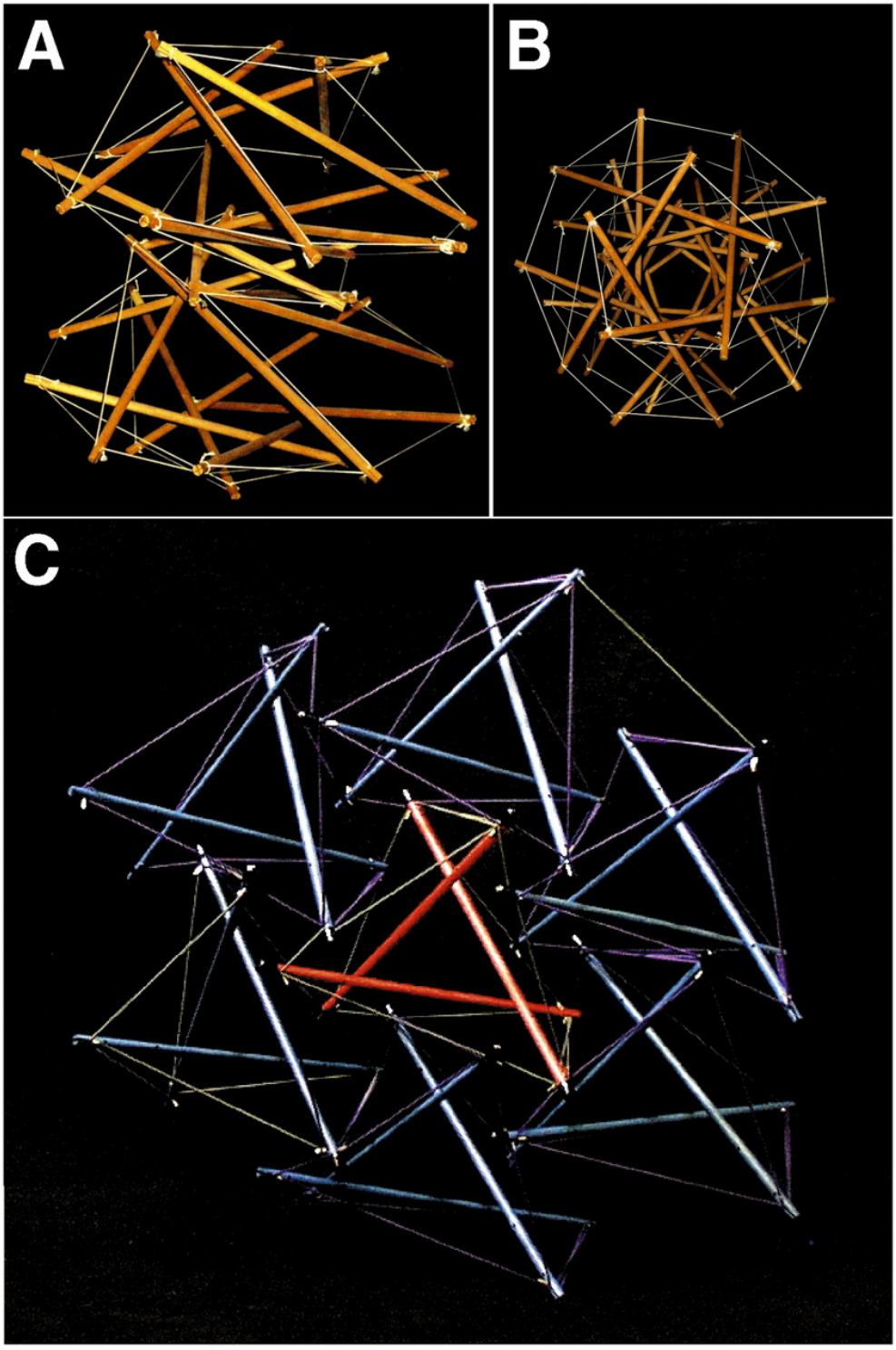

Fig. 7. Multimodular tensegrities. (A) A side view of a tensegrity structure composed of four interconnected modules which each contain five struts. (B) A top view of the tensegrity structure shown in A, showing five-fold symmetry and a central pore. (C) A tensegrity lattice comprising seven similar tensegrity modules; a single three-strut module is shown in red.

Computer simulations of complex multimodular tensegrity arrangements depict subtle mechanical behaviors that are reminiscent of those of living cells. For example, a simulation of a prestressed fabric composed of multiple interconnected tensegrity modules displays coordinated retraction of all the support elements throughout the depth of the material when it is released from its anchors (Fig. 8A). This response is similar to what happens to the cell, cytoplasm and nucleus following addition of trypsin to cleave ECM anchors (Fig. 8B) or to whole living tissues (e.g. skin or muscle) following a surgical incision. Another computer simulation revealed that, when physically extended, a fabric comprised of multiple (36) interconnected tensegrity modules (each containing 6 struts and 24 cables, as in Fig. 1B) displayed undulating movements (Fig. 8C) that are similar to those exhibited by extending lamellipodia in living cells (Fig. 8D). This observation raises the possibility that the actin filaments that rapidly polymerize (elongate) within a newly forming lamellipodium push out against the surrounding actin filament network and surface membrane and thereby prestress the entire structure. It also may explain why lamellipodia generally exhibit a similar morphology in all cells: their form is a manifestation of the underlying force balance that stabilizes their three-dimensional architecture and not a direct property of any one of its individual components. The observations that directional movement of the cytoplasm is controlled through a balance between cytoskeleton-based protrusive and retractive forces (Verkhovsky et al., 1999), decreasing the tension (stiffness) in the surface membrane accelerates lamellipodia extension (Raucher and Sheetz, 2000), and rapid linear extension of acrosomal processes is based on a dynamic balance between extension of rigid actin struts and resisting membrane elements (Tilney and Inoue, 1982) also support the generality of this model for movement of subcellular microdomains.

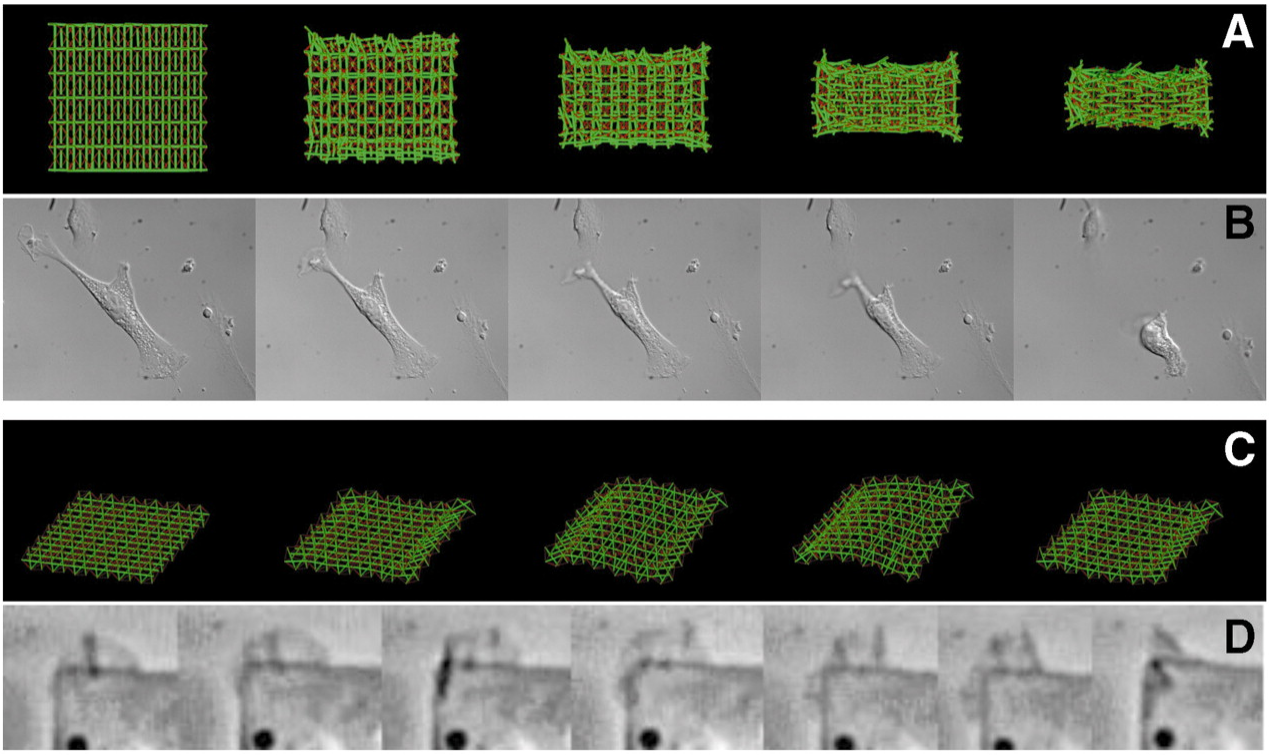

Fig. 8. Sequential images (left to right) from computer simulations of multimodular tensegrities (A,C) or from time-lapse video recording of living cells (B,D). (A) Structural rearrangements within a prestressed tensegrity lattice immediately following release of its anchors (at the top and bottom of the view). Note that the material simultaneously retracts throughout its entire depth. (B) When the ECM adhesions of a spread, adherent cell are dislodged using trypsin, the cell, cytoplasm and nucleus all simultaneously retract as the cell rounds (left to right). (C) A prestressed tensegrity fabric created from 36 interconnected tensegrity modules of the type shown in Fig. 1B that experiences a distending force at the top right corner; the other three corners are fixed. Notice that the entire material responds to the local force and that it exhibits undulating motion. (D) Undulating motion of a lamellipodium in a living cell.

Implications for the hierarchical nature of biological systems

Importantly, the cellular tensegrity model also takes into account the hierarhical features of living cells as well as those of the tissues and organs in which they normally reside (Ingber and Jamieson, 1985; Ingber, 1993b; Ingber et al., 1994; Ingber, 1998). This level of complexity is commonly ignored in cell biology. Fuller was the first to note that tensegrity systems can be constructed as structural hierarchies in which the tension or compression elements that comprise the structure at one level are themselves tensegrity systems composed of multiple components on a smaller scale (Fuller, 1961). The

tensegrity model of the nucleated cell, in which the entire nuclear tensegrity lattice is itself a tension element in the larger structure (Fig. 4B), illustrates this concept.

Living organisms are similarly constructed as tiers of systems within systems within systems. The bones and muscles of our bodies use a tensegrity force balance to stabilize themselves (Levin, 1997; Chen and Ingber, 1999). Whole organs, such as the heart and lung, are also prestressed structures (Omens and Fung, 1990), owing to tension generation within their constituent cells and the existence of larger-scale distending forces (e.g. hemodynamic forces and air pressure). Neural architecture in the brain (Van Essen, 1997) and retina (Galli-Resta, 2002) are also governed by internal tissue forces, in this case generated within the cytoskeletons of their constituent cells. The forces in these tissues and organs are resisted by stiffened ECMs (e.g. crosslinked collagen bundles, elastin bundles and basement membranes), by the non-compressibility of proteoglycan-rich ECMs and other cells, and by opposing contractile forces generated by neighboring cells (e.g. mesenchyme versus epithelium). It is for this reason that the edges of the wound spontaneously retract when a tissue or organ is incised with a scalpel (Liu and Fung, 1989; Omens and Fung, 1990).

A counterintuitive feature of hierarchical tensegrity structures is that a tensed member on one size scale can act locally to resist compression on a smaller size scale. A simple analogy is how rats can climb up a ship's mooring rope by compressing it locally between their front and rear feet, but only when the rope is tensionally stiffened. Similarly, the existence of a stabilizing prestress in a whole organ or tissue stiffens internal tension elements, such as basement membranes, which, in turn, may resist compression applied locally by individual adherent cells (i.e. between their isolated focal adhesions) and thereby stabilize cell shape on the microscale.

But the tensegrity hierarchy does not end at the level of the cell. The internal cytoskeleton that behaves like a tensegrity structure also connects to the elastic submembranous cytoskeleton at the cell periphery and to the nuclear scaffold at the cell center (Fey et al., 1984; Georgatos and Blobel, 1987; Maniotis et al., 1997a; Zhen et al., 2002). At the molecular level, the submembranous cytoskeleton is another tensegrity structure: it is a discrete network composed of actin, ankryin and spectrin molecules that is both prestressed (Discher et al., 1998), owing to transmembrane osmotic forces, and organized geodesically within a hexagonal network (Liu et al., 1987). The entire network and attached membrane undergo expansion and contraction in response to changes in osmotic pressure. Although this is mediated by elongation of individual molecules in the network, such as spectrin, the geodesic arrangement might also facilitate this process by permitting these large-scale shape changes without disruption of network continuity (e.g. breakage of individual struts). This capability of geodesic structures is visualized in Fig. 9, which shows a geodesic sphere created by the designer Chuck Hoberman that undergoes large-scale expansion and contraction by using a kinematic mechanism to produce elongation of individual network members, rather than molecular distortion as in living cells. In fact, as described by Caspar, it may be because of tensegrity that geodesic viral capsids can similarly expand and contract without loss of structural integrity (Caspar, 1980).

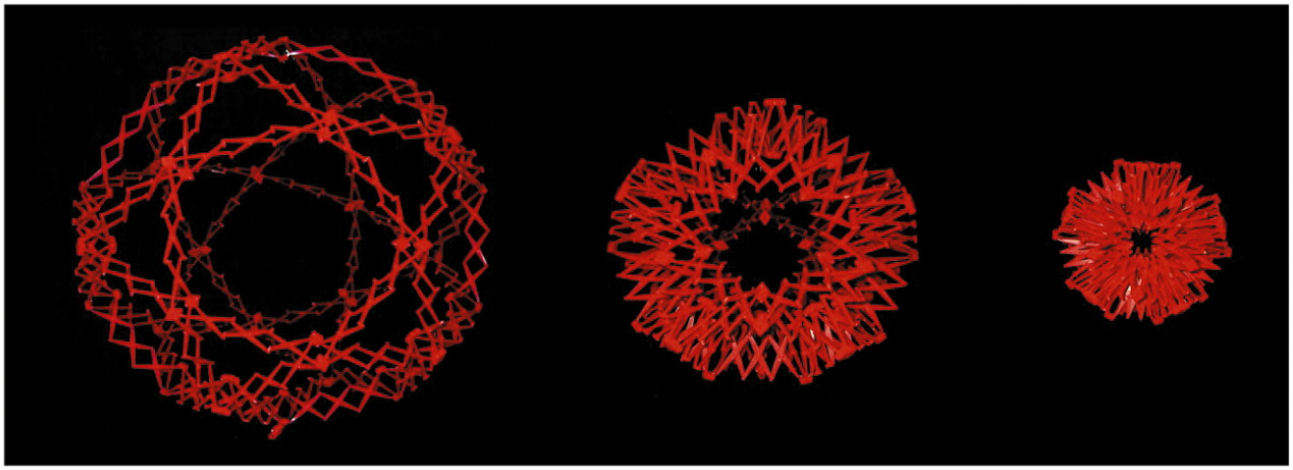

Fig. 9. Visualization of expansion and contraction behavior through use of a geodesically structured support network using the Hoberman Sphere created by the designer, Chuck Hoberman (Hoberman Toys, Inc.). This single structure, which is shown in three states of expansion in this figure, uses scissor-like struts that extend in a coordinated manner via a kinematic mechanism to provide large-scale shape changes in the entire structure without disrupting network integrity. In geodesic molecular networks, such as the submembranous cytoskeleton or viral capsids, extension is largely driven by molecular shape changes (e.g. elongation of individual spectrin molecules or viral proteins).

The nucleus may represent yet another tensegrity structure (Ingber and Jamieson, 1985; Ingber, 1993b; Ingber et al., 1994), because it is prestressed and exhibits shape stability even when isolated from the cell (e.g. during nuclear transplantation). During mitosis, microtubule struts polymerize from two centrosomes oriented at opposite poles of the cell and push out against a mechanically continuous network of chromatin (Maniotis et al., 1997b), thereby creating the `mitotic spindle' that holds the chromosomes in position. Laser microbeam experiments have confirmed that this tensionally stiffened spindle is a prestressed tensegrity cage (Pickett-Heaps et al., 1997). What maintains nuclear shape in interphase cells is less clear; however, there is no doubt that the nucleus is prestressed: cleave the protein lattice that makes up the nuclear matrix and the tightly packaged (compressed) DNA explodes outward. Nuclear shape stability in the living cell, however, also depends on the presence of tensed intermediate filaments that connect the nucleus to cell-surface adhesions and thus act like molecular guy wires at the level of the whole cell (Maniotis et al., 1997a). These different subcellular tensegrity structures (e.g. the internal cytoskeleton, submembranous cytoskeleton and nucleus) may act independently, but when mechanically coupled they function as one integrated, hierarchical tensegrity system.

On a smaller scale, cells also use a tensegrity force balance to stabilize the elongated forms of specialized membrane projections. Stiffened bundles of crosslinked actin filaments push out on the tensed surface membrane to create filopodia that extend from the cell surface at the leading edge of migratory cells (Sheetz et al., 1992) and to form

acrosomal extensions in sperm (Tilney and Inoue, 1982). Crosslinking of any type of flexible molecular filament into larger bundles greatly increases its ability to resist compression because the fixed lateral connections prevent filament buckling or bending, just as a metal hoop stiffens wood struts in a barrel. Thus, microfilaments, which normally bear tension in the cell, have a dual function in that they can act as compression struts when organized in this manner. Crosslinked bundles of microtubules similarly stabilize cilia as well as long cell processes, as in neurites (Joshi et al., 1985).

Prestressed and geodesic forms of tensegrity also occur at the molecular level. The most impressive example of a geodesic form is the finding that actin microfilaments self-organize into well developed geodesic domes (actin geodomes) in the cytoskeletons of certain cells in vitro (Fig. 3B) (Lazarides, 1976; Osborn et al., 1978) as well as in vivo (Rafferty and Scholtz, 1985). Other examples of geodesic structures include hexagonal arrangements of basement membrane proteins (Yurchenco and Schittny, 1990), polyhedral enzyme complexes (Wagenknecht et al., 1991), clathrin-coated transport vesicles (Vigers et al., 1986) and all viral capsids (Caspar, 1980). Biological polymers, such as microfilaments (Schutt et al., 1997), lipid micelles (Butcher and Lamb, 1984; Farrell et al., 2002), and individual proteins, RNA and DNA molecules all have been depicted as prestressed tensegrity structures (Ingber, 1998; Ingber, 2000b; Farell et al., 2002) because at this scale no components `touch' and, hence, all structural stability must depend on continuous tensional (attractive) forces. For example, in proteins, stiffened peptide elements (e.g. α-helices and ß-strands) act locally to resist inwardly directed forces generated by attractive (tensile) intramolecular binding forces. Thus, three-dimensional models of the shape of a protein, such as a membrane channel, are not unlike tensegrity models (Fig. 7A,B) composed entirely of springs that have different elasticities (as in Fig. 1C); the major difference is that that intramolecular binding forces obviate the need for physical tensile connections in the proteins. The prestressed nature of proteins can be visualized if a single peptide bond is cleaved: immediate loss of shape stability results. Moreover, studies with optical tweezers reveal that individual DNA molecules exhibit linear stiffening behavior (Smith et al., 1992) similar to that of living cells, tissues and tensegrity models.

For these reasons, the cellular tensegrity model has come to include the concept that cells, tissues and other biological structures at smaller and larger size scales exhibit integrated mechanical behavior because of tensegrity architecture (Ingber and Jamieson, 1985; Ingber, 1993b; Ingber, 1998; Pienta and Coffey, 1991; Pienta et al., 1991a; Ingber et al., 1994). The recognition that nature uses both prestressed and geodesic structures at smaller size scales in the cell also provides further evidence to suggest that these different classes of structure are manifestations of a common "design" principle. Geodesic tensegrity forms (e.g. tetrahedra, octahedra and icosahedra) similarly predominate in the inorganic world of crystals and atoms and thus, this principle may have contributed to how life first emerged on this planet (Ingber, 2000b).

Conclusion

In Part I of this Commentary, I have reviewed results from many studies carried out over the past decade that provide strong evidence in support of the cellular tensegrity model. Importantly, any one of these findings is not sufficient to prove the tensegrity theory and some (e.g. strain hardening behavior) may even be explained equally well by other approaches (Heidemann et al., 2000). However, the prestressed tensegrity model of the cell is the only existing theory of cell structure that provides a unified way to explain all of these results. It is also important to note that there is a difference between a `computational model', which may simply be an ad hoc calculation based on known data (or data estimates), versus a mathematical formulation of a theory, which uses computational approaches to test a priori predictions of the model. Essentially all past modeling work on cell mechanics involves the former, whereas the results with the tensegrity model represent the latter.

The power of the tensegrity theory to predict complex cell behaviors from first principles, to mimic pattern formation within the cytoskeleton on the nanoscale and to translate cell shape control into molecular terms speaks for itself. Yet, for many molecular cell biologists, there is still little value in this knowledge. They do not need to take into account the contributions of physical forces or supramolecular assemblies in studies that focus on individual molecules or signaling mechanisms. However, at some point, we all will have to translate what we have learned from our simplified systems in order to predict, manipulate and control cellular function in vivo. Then physical factors, tissue structure and understanding of hierarchical systems biology — how molecular processes function within living multicellular organisms — will become important.

For those interested in cell and tissue physiology, cell context is already critical. Pursuit of the tensegrity model has led to new insights into cell mechanics and to the recognition that mechanical stresses can be transferred through the viscous cytosol and to the nucleus in living cells through discrete molecular networks. It also has helped to explain how living organisms can function as integrated mechanical systems, even though they are complex hierarchical structures (molecules within cells within tissues within organs). Indeed, the tensegrity principle has been invoked by investigators to explain an unusually wide range of unexplained phenomena in many different systems and species, including: lipid micelle formation (Butcher and Lamb, 1984), protein folding in milk globules (Farrell et al., 2002), protein organization within viral capsids (Caspar, 1980), the structure of actin microfilaments (Schutt et al., 1997), pattern formation in paramecium (Kaczanowska et al., 1995), hyphal morphology in fungi (Kaminsky and Heath, 1996), neurite outgrowth (Joshi et al., 1985; Buxbaum and Heidemann, 1988), endothelial permeability barrier function (Moy et al., 1998), vascular tone (Northover and Northover, 1993), dystrophin function in muscular dystrophy (Gillis, 1999), choriocarcinoma differentiation (Hohn et al., 1996), control of apoptosis (Ciesla, 2001), morphogenesis of mammalian cells and tissues (Ingber et al., 1981; Ingber and Jamieson, 1985; Pienta and Coffey, 1991a; Pienta et al., 1991; Huang and Ingber, 1999; Ingber, 1993; Ingber et al., 1994), the structure of the skin (Ryan, 1989), lens

(Yamada et al., 2000), cartilage (Malinin and Malinin, 1999), retina (Galli-Resta, 2002) and brain (Van Essen, 1997), the mechanics of the human skeleton (Levin, 1997), tumor formation and metastasis (Ingber et al., 1981; Ingber and Jamieson, 1985; Pienta and Coffey, 1991b; Huang and Ingber, 1999), as well as gravity sensing in both animals and plants (Ingber, 1999; Yoder et al., 2001). In addition, it has helped to elucidate the molecular basis of cellular mechanotransduction and has revealed previously unrecognized roles of the ECM, cytoskeletal structure and cytoskeletal tension (prestress) in the control of cellular information processing, as I will describe in Part II of this Commentary (Ingber, 2003).

The cellular tensegrity model remains a work in progress that will continue to be refined as more information emerges. However, the ability of the tensegrity theory to predict and explain complex cell behaviors is a testament to the notion posed by D'Arcy Thompson in the quote that opens this article (Thompson, 1952): although the living cell is a complicated structure, it still may be governed by simple rules.

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