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Models of cytoskeletal mechanics of adherent cells

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Abstract Adherent cells sense their mechanical environment, which, in turn, regulates their functions. During the past decade, a growing body of evidence has indicated that a deformable, solid-state intracellular structure known as the cytoskeleton (CSK) plays a major role in transmitting and distributing mechanical stresses within the cell as well as in their conversion into a chemical response. Therefore in order to understand mechanical regulation and control of cellular functions, one needs to understand mechanisms that determine how the CSK changes its shape and mechanics in response to stress. In this survey, we examined commonly used structurally based models of the CSK. In particular, we focused on two classes of these models: open-cell foam networks and stress-supported structures. We identified the underlying mechanisms that determine deformability of those models and compare model predictions with data previously obtained from mechanical tests on cultured living adherent cells at steady state. We concluded that stress-supported structures appear more suitable for describing cell deformability because this class of structures can explain the central role that the cytoskeletal contractile prestress plays in cellular mechanics.

1
Introduction

It is well established that alterations of cell shape caused by mechanical loads affect a host of cellular functions including locomotion, growth differentiation and proliferation (cf., Harris et al. 1980; Dembo 1989; Singhvi et al. 1994; Pelham and Wang 1997; Chen et al. 1997; Chicurel et al. 1998; Janmey 1998). Therefore, the central question of cellular mechanics is: By what mechanisms do cells resist shape distortion and maintain their structural stability? For many years a standard paradigm has been that the cortical membrane that surrounds the liquid cytoplasm provides cell shape stability. Although this model has been proven useful in studies of suspended cells (e.g., blood cells) (Evans and Yeung 1989), it has enjoyed a limited success in describing deformability of adherent cells (e.g., endothelial cells, smooth-muscle cells, epithelial cells, fibroblasts). During the last decade, a growing body of evidence has shown that an intracellular molecular framework, known as the cytoskeleton (CSK), plays a major role in transmitting mechanical stresses from the cell surface, across the cytoplasm and into the nucleus (Ingber 1993; Wang et al. 1993; Davies and Tripathi 1993; Maniotis et al. 1997; Wang et al. 2001). Accordingly, more recent studies in cellular mechanics have focused on revealing the role of the CSK in the mechanism by which cells resist shape distortion. The goal of this article is to review major approaches and key accomplishments in CSK mechanics from a structural mechanical point of view.

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Cytoskeleton

The CSK is an interconnected structure of various cross-linked and interlinked filamentous biopolymers that extends from the nucleus to the cell surface. Three major filamentous biopolymers comprise the CSK: actin microfilaments, microtubules and intermediate filaments (cf., Amos and Amos 1991). Mechanical roles of these three filamentous systems have not yet been fully understood, but experimental studies in living cells as well as measurements in vitro have revealed some insights into their function.

The actin network of the CSK is comprised of little extensible cross-linked filaments (diameter of 5–10 nm, Young's modulus order of GPa) (cf., Gittes et al. 1993). The primary role of these filaments is to carry tensile forces that are generated actively by the cell's contractile apparatus (i.e., driven via acto-myosin interactions) and also passively by cell distention through its adhesive substrate or by the swelling pressure (turgor) of the liquid cytoplasm (cf., Ingber 1993). Other actin structures include stress fibers, which are bundles of actin filaments that, according to recent findings, can carry both tensile and compressive forces (Hucker et al. 1999). Microtubules are tubular biopolymers (outer and inner diameters of ~24 and ~12 nm, respectively, Young's modulus order of GPa) (cf., Gittes et al. 1993) that appear to carry compressive forces as they resist contraction of the interconnected actin network (Kolodney and Wysolmerski 1992; Waterman-Storer and Salmon 1997; Putnam et al. 1998; Wang et al. 2001; Stamenoviæ et al. 2002b). The role of the intermediate filament network in living cells is less understood than the roles of the other two networks. Intermediate filaments (diameter of ~10 nm) are believed to carry tension, but only at relatively large applied strains (>20%) when their contribution to the cell's rigidity becomes significant (Janmey et al. 1991: Wang and Stamenoviæ 2000). These three filamentous networks of the CSK are physically interlinked (cf., Svitkina et al. 1996) which facilitates force transmission between them.

Another important component in the cellular force–balance milieu is the extracellular matrix (ECM). A portion of the mechanical forces within the CSK is transferred to the ECM via transmembrane integrin receptors that concentrate in focal adhesion plaques (Wang et al. 1993). As a consequence, traction forces arise at the cell–ECM interface. These traction forces together with the forces within the CSK form a self-equilibrated, stable mechanical system. For that very reason, Ingber (1993) has referred to the ECM as the "extended CSK". Importantly, many of the enzymes and substrates that mediate critical cell functions, including DNA synthesis, RNA processing, glycolysis, signal transduction, and ECM remodeling physically associate with the insoluble molecular scaffolds that comprise the CSK and ECM (Chicurel et al. 1998). Thus, understanding how the three-dimensional form of the CSK is stabilized has fundamental implications for understanding how living cells sense and respond to mechanical stresses.

Two classes of structural mechanical models of the CSK have been advanced to elucidate potential mechanisms by which adherent cells develop mechanical stresses in order to resist shape distortion. In one, stress within the CSK arises primarily from deformation of individual CSK filaments (e.g. stretching, bending, and torsion) under the action of externally applied load to the cell (Satcher and Dewey 1996). These models are known as open-cell foams. In the other, there is a pre-existing mechanical stress ("prestress") within the CSK that plays the central role in resisting applied loads. This class of models is known as stress-supported structures (cf., Stamenoviæ and Coughlin 1999; Wang et al. 2002).

In this paper, we review major microstructural models, both open-cell foams and stress-supported structures that have been used in the past to describe elastic behavior of adherent cells. We also examine alternative, not structurally based models. Before reviewing various models of the CSK, we offer a brief survey of major results from experimental measurements that are relevant for understanding CSK mechanics.

2
Results from measurements of living cells

Results from mechanical analysis of various types of living adherent cells, obtained by many different experimental techniques, reveal several distinct features. First, cell rheological behavior is apparently viscoelastic; cells exhibit creep (cf., Sato et al. 1990; Wang and Ingber 1995; Bausch et al. 1998), stress relaxation (cf., Thoumine and Ott 1997) and hysteresis (cf., Petersen et al. 1982; Maksym et al. 2000; Fabry et al. 2001). However, indices of cell mechanical properties (elastic moduli, apparent viscosity, time constants) determined from those measurements are highly scattered. For example, values of measured elastic shear modulus of the cell range from as low as $O(10^0)$ Pa up to $O(10^5)$ Pa (cf., Stamenoviæ and

Coughlin 1999). This wide range of values reflects primarily methodological differences between the various experimental techniques used (e.g., size of probe, lack of molecular specificity) rather than the type of cells examined. However, several different techniques yielded values of elastic shear modulus of adherent cells that fall within or very close to the range of $O(10^2)$ to $O(10^3)$ Pa (Petersen et al. 1982; Sato et al. 1990; Thoumine and Ott 1997; Maksym et al. 2000; Fabry et al. 2001). Thus, we believe that it is reasonable to assume that true steady-state shear modulus of adherent cells falls within this range. An important feature of the cell mechanical behavior is the observed prestress-induced stiffening. An increase in the contractile stress within the CSK or an increase in cell distension is paralleled by an increase in cell stiffness (Wang and Ingber 1994; Pourati et al. 1998; Wang et al. 2002). For example, it is found that in living airway smooth-muscle cells, CSK stiffness increases linearly with increasing contractile stress (Wang et al. 2001, 2002). This, in turn, suggests that the CSK is organized as a stress-supported structure, i.e., a structure that carries pre-existing stress (prestress) prior to application of applied forces. A positive dependence of structural stiffness on the prestress is a key feature of this class of structures (cf., Volokh and Vilnay 1997).

Cells exhibit strain hardening, i.e., cell stiffness increases progressively in response to externally applied load (cf., Petersen et al. 1982; Sato et al. 1990; Wang et al. 1993; Shroff et al. 1995; Radmacher 1997). These, in turn, indicate that cells exhibit a higher than linear stress–strain behavior. However, it has been shown recently that in some measurements a part of the observed strain hardening is due to an artifact (Fabry et al. 1999).

Dynamic behavior of adherent cells is characterized by a wide spectrum of time constants; both elastic (storage) and frictional (loss) moduli exhibit a weak power dependence on frequency over a wide frequency range (10^{-2} to 10^3 Hz) (Fabry et al. 2001). This feature may not be a reflection of particular molecular mechanisms, rather than a reflection of some higher-level structural organization (Sollich 1998). Another property of dynamic elastic and frictional moduli is that both increase linearly with increasing level of CSK prestress (Stamenoviæ et al. 2002a).

Observations of the deformation of CSK structures in living cells using various imaging techniques reveal several important features. First, pushing and pulling of a glass micropipette whose tip was bound to integrin receptors at the surface of endothelial cells (these trans-membrane receptors are directly linked to the actin network of the CSK) caused coordinated deformation of both the CSK and the nucleus (Maniotis et al. 1997). A similar experiment was done in endothelial cells in which mitochondria were fluorescently labeled; mitochondria directly associate with microtubules. When stress was applied directly to integrin receptors, coordinated movement and alignment of mitochondria throughout the cytoplasmic region were observed (Wang et al. 2001). Taken together these observations suggest that internal cell deformation depends on the molecular connectivity of CSK filaments from the cell surface to the nucleus. This, in turn, implies that the model that depicts the cell is an elastic membrane that surrounds a liquid cytoplasm is not consistent with these observations. Second, in epithelial and endothelial cells fluorescent-labeled microtubules buckle in response to cell contraction (Waterman-Storer and Salmon 1997; Wang et al. 2001). This, in turn, suggests that microtubules carry compression as they balance contractile stress carried by the actin network.

3
Mechanical models of cell deformability

Despite its complex and dynamic architecture, the CSK is usually modeled assuming relatively simple, idealized structural geometries, assuming material isotropy, homogeneity, and elasticity. The idea is that if the mechanisms by which those various idealized models develop mechanical stresses are indeed embodied within the CSK, then despite all those simplifications the model should be able to capture key features that characterize mechanical behavior of the cell. In this section we examine two major types of structurally based models of the CSK: open-cell foams and stress-supported structures.

3.1
Open-cell foams

These are networks of interconnected struts. If the relative foam density φ (i.e., the volume fraction of struts relative to the volume of the foam, $0 < \varphi < 1$) is very small, then the structural elastic modulus is proportional to either φ or φ^2 depending on whether stretching or bending and twisting of the struts, respectively, are major modes by which the structure develops restoring stress in response to the applied load (cf., Warren and Kraynik 1997). The

relative foam density can be obtained as the ratio of foam mass density to the mass density of an individual strut (Satcher and Dewey 1996).

Satcher and co-workers (1996, 1997) used open-cell foams as a model of the actin CSK of cultured endothelial cells (Fig. 1). They assumed that bending and twisting of actin filaments is the basic mode by which the actin network develops mechanical stress. Their assumption was based on apparent similarity between the actin network in endothelial cells and microstructural networks of various natural and synthetic materials that are known to resist distortion by bending of their structural components. Using the approach of Gibson and Ashby (1982), they obtained the general formula for the shear modulus (G) of such materials as follows (for derivation, see the caption under Fig. 1):

$$G \propto E_f \varphi^2 \tag{1}$$

where E_f is Young's modulus of individual struts. Taking the values of $\varphi \approx O(10^{-3})$ and $E_f = O(10^9)$ Pa for actin from the literature (Satcher et al. 1997; Gittes et al. 1993), it follows from Eq. (1) that the shear modulus of the actin network of the CSK is $G \approx O(10^3)$ Pa. This value is at the very upper bound of the reliable range of measured shear moduli [$O(10^2$–$10^3)$ Pa] of cultured adherent cells. In addition, the open-cell foam model predicts strain hardening during compression (Gibson and Ashby 1982), a feature which is consistent with the observed strain hardening of cultured adherent cells exposed to local indentation (cf., Petersen et al. 1982; Shroff et al. 1995; Radmacher 1997).

We also considered the possibility that the microtubules form an open-cell foam network (Stamenoviæ and Coughlin 1999). Since the values of φ and the Young's modulus for microtubules are of the same order of magnitude as in the actin network, the predicted value of G is also similar.

An open-cell foam model where the principal modes of resistance to applied external load include stretching of individual structural components would lead to an overprediction of G. In such a model $G \propto E_f \varphi$ (cf., Warren and Kraynik 1997) and since $\varphi \ll 1$, G would be much greater than the values predicted by Eq. (1).

It is important to note that none of these open-cell models includes prestress. As such, they cannot explain the observed dependence of cell stiffness on CSK prestress.

Based on the above, we concluded that open-cell foams have a relatively limited application as models of the CSK.

3.2
Stress-supported structures

Stress-supported or prestressed structures are networks that require pre-existing tensile stress or prestress in their structural components in order to maintain their structural stability. The

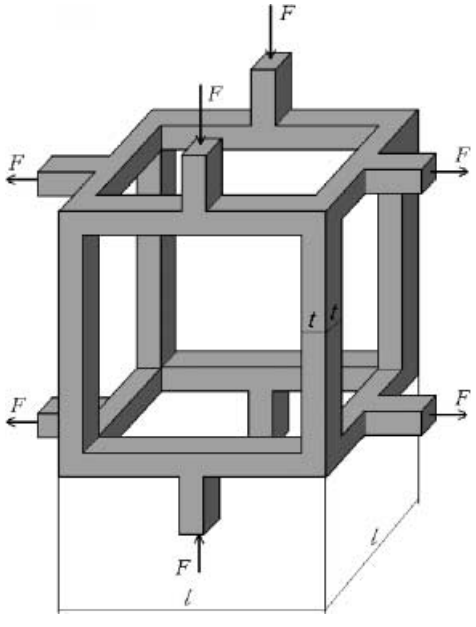

Fig. 1. The representative structural unit of the open-cell foam network used by Satcher and Dewey (1996) to describe cytoskeletal mechanics of endothelial cells. The unit is made of elastic beams of length l and thickness t, which bend under force F generating thereby recoil stress that opposes the applied load. In the analysis of Satcher and Dewey (1996) that connects the micro- and macro-mechanics of the open-cell foam model, only proportional ($\propto$) relationships are considered. The center deflection of the beam is $u \propto Fl^3/E_f I$ where E_f is Young's modulus and I is moment of inertia of the beams, $I \propto t^4$. The applied shear stress to the network is $s \propto F/l^2$ and the shear strain is modulus $\gamma \propto u/l$. The shear modulus $G = s/\gamma$. By combining these relationships it follows that $G \propto E_f(t/l)^4$. Since the volumetric fraction of the beams is $\varphi \propto (t/l)^2$, it follows that $G \propto E_f \varphi^2$

greater the prestress, the more stable and therefore the stiffer these structures are. In the absence of the prestress these structures lose their stability and collapse. Examples of such structures include spider webs, pup tents, plant leafs, and gas–liquid foams. All these structures share common mechanisms by which they develop mechanical stress to oppose shape distortion: change in spacing, change in orientation, and expansion and contraction of their structural members. The only difference between various types of those structures is the manner by which they balance the prestress. In some structures the prestress is entirely balanced by the structure's attachments to the external objects (e.g., tree branches in the case of spider webs). In others, the prestress is entirely balanced by internal elements (e.g., like pressurized air in bubbles that balances surface tension in gas–liquid foams). There are structures where the prestress is balanced both internally and externally (like struts versus pegs in pup tents). A special class of structures where the prestress is balanced by compression-supporting elements is so-called tensegrity structures that will be considered later in the text. A distinct feature of all stress-supported structures is that their shear modulus increases approximately linearly with increasing prestress, i.e., they exhibit a prestress-induced hardening (Stamenoviæ and Wang 2000). The most commonly used stress-supported structures in cellular mechanics include the cortical membrane model and tensed cable networks with the tensegrity model as the most prominent among the latter.

Cortical membrane model

This model rests on the assumption that the main force-bearing structures of the cell are confined within a thin (~100 nm) cortical layer (Zhelev et al. 1994) or several distinct layers (Heidemann et al. 1999). The membrane encloses a liquid cytoplasm. The membrane is under sustained tension that is either entirely balanced by the pressurized cytoplasm in suspended cells, or partly by the cytoplasm and partly by traction at the extracellular adhesions in adherent cells. The model has been successful in describing mechanical properties of suspended cells (blood cells, see urchin eggs) (Hiramoto 1963; Evans and Yeung 1989; Discher et al. 1998) where the cortical layer rich in actin and spectrin is distinct from the liquid cytoplasmic domain. Later on it was adopted as a model of adherent cells (Fung and Liu 1993; Schmid-Schönbein et al. 1995; Heidemann et al. 1999). An example of application of this model to adherent cells is given below (Stamenoviæ and Wang 2000).

The model is depicted as a cortical membrane of thickness $h \approx 0.1$ μm that surrounds a liquid cytoplasm. The membrane is under sustained contractile stress (prestress) that is balanced by traction at the cell–substrate interface and by the pressure of engulfed cytoplasm. Measurements on adherent airway smooth-muscle cells show that traction forces balance nearly 90% of the prestress (Stamenoviæ et al. 2002b) and thus we omitted the contribution of cytoplasmic pressure from further considerations. The membrane prestress (σ) was obtained from traction (τ) measurements by considering a free-body diagram of a section of the cell, $\sigma = \tau A'/A''$ where A' and A'' are cell–substrate interfacial area and membrane cross-sectional area, respectively (Fig. 2a). Based on experimental data for maximal traction in non-stimulated airway smooth-muscle cells (Wang et al. 2002), we estimated the membrane prestress $\sigma = O(10^4)$ Pa. Magnetic twisting measurements, in which small ferromagnetic beads bound to cell surface integrin receptors are twisted to probe mechanical properties of the cell and

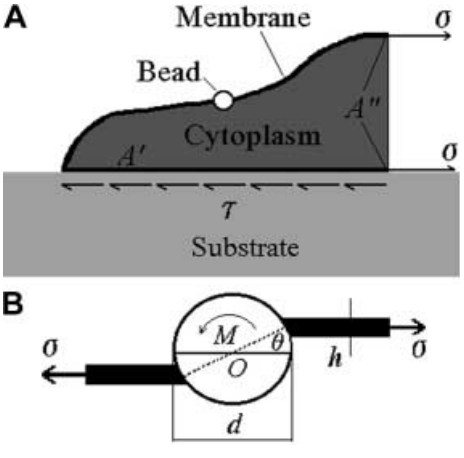

Fig. 2. **A** A free-body diagram of a section of the cortical membrane model. Traction (τ) at the cell–substrate interface is balanced by the cortical tensile stress (σ): $\tau A' = \sigma A''$ where A' and A'' are interfacial cell–substrate area and cross-sectional area of the cortical membrane, respectively. A spherical bead embedded in the membrane simulates magnetic twisting cytometry measurements (Wang and Ingber 1994). **B** A free-body diagram of the bead. Applied twisting torque M is balanced by cortical tension σ. $M = \sigma d^2 h \sin\theta$ where d is bead diameter, h is thickness of the cortical membrane and θ is the angle of rotation of the bead around its center O

internal CSK (Wang et al. 1993), were simulated as follows. A rigid spherical bead of diameter $d = 4.5$ μm is embedded in the tensed membrane (Fig. 2a). A twisting torque M is applied to the sphere in the vertical plane. Rotation of the sphere is impeded by the membrane tension σ (Fig. 2b). The contribution of cytoplasmic viscosity is not taken into account since we consider only static equilibrium. From the mechanical balance it follows that:

$$M = \sigma d^2 h \sin\theta \tag{2}$$

where θ is the angle of bead rotation (angular strain). Defining applied stress as the ratio of M and 6×bead volume, where 6 is the shape factor, and shear stiffness G as the ratio of such obtained stress and the corresponding θ (Wang et al. 1993; Wang and Ingber 1994), it follows from Eq. (2) that:

$$G = \frac{1}{\pi}\sigma\frac{h}{d}\frac{\sin\theta}{\theta} \quad . \tag{3}$$

The apparent shear modulus is obtained from Eq. (3) in the limit of $\theta \to 0$, $G \to (1/\pi)\sigma(h/d)$. Thus, for a given θ, G increases linearly with σ, which is consistent with experimental observations (Wang et al. 2002). Taking into account the experimental values of σ, h, and d it follows that the $G = O(10^2)$ Pa which is at the lower bound of the acceptable range of experimental values of G at steady state. According to Eq. (3), G decreases with increasing θ, which indicates softening whereas experimental data show that during magnetic twisting measurements cell exhibit hardening (Wang et al. 1993; Wang and Ingber 1994). Equation (3) also predicts that G decreases with increasing bead diameter d, whereas experimental data show quite the opposite trend (Wang and Ingber 1994).

Despite some good agreements of this model with experimental data, we are only moderately enthusiastic about its applicability to CSK mechanics of adherent cells. The main reason is the intrinsic assumption of this model that cell resistance to shape distortion is entirely provided by a thin, elastic cortical layer(s) that surrounds liquid cytoplasm. This assumption contradicts observations that the mechanical perturbations exerted on the surface of adherent cells are transmitted deep into the cytoplasmic domain and to the nucleus, and that this transmission is facilitated by molecular connectivity of the CSK and rearrangements within the microtubule lattice (Maniotis et al. 1997; Wang et al. 2001). However, this model may be useful to explain mechanical behavior of suspended cells, including non-adherent suspended cells, where the CSK appears to be organized primarily as a thin cortical layer of actin (Sato et al. 1987).

Tensed cable networks

These are reticulated structures composed of tensile elements (cables), which cannot support compression. Cables carry initial tension thereby conferring shape stability to the structure. The initial tension defines the prestress as described below. Balance to the cable tension is provided either externally (e.g., by the ECM), internally (e.g., by compression-supporting elements of the CSK or by pressurized cytoplasm), or by a combination of the two. In the CSK, tensed actin filaments are viewed as playing the role of cables (Stamenoviæ and Coughlin 1999). Regardless of how cable tension is balanced (internally, externally or by combination of both), the prestress (P) can be defined as the sum of all tensile forces transmitted by cables across an arbitrary cross-sectional area (A) of the cell per unit area before application of external loads (Fig. 3):

$$P = \frac{\sum_{i=1}^{n} F_i \cos\theta_i}{A} = \frac{n\langle F\cos\theta\rangle}{A} \tag{4}$$

where F_i is tensile force in the ith cable, θ_i is the corresponding angle with respect to the outer normal vector to A, n is the number of cables intersected by A and $\langle\cdot\rangle$ denotes the average over all fiber orientations (Stamenoviæ and Coughlin 1999). In the case that all cables carry the same initial tension and that their orientations are equally probable it follows that $n\langle F\cos\theta\rangle/A = \varphi\sigma_c/3$ (Stamenoviæ and Wilson 1992) where φ is the relative density of the cables in the structure and σ_c is the tensile stress in the cables obtained as the ratio of the

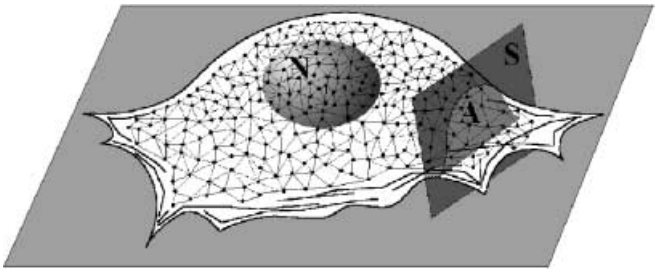

Fig. 3. A schematic depiction of the actin network in adherent cells with the nucleus *N*. A surface (*S*) intersects the network. The net tensile forces transmitted across the cross-sectional area (*A*) by initially tensed actin filaments per unit area define the prestress (Eq. 4)

tensile force per unit reference cross-sectional area of the cable. Thus it follows from Eq. (4) that:

$$P = \frac{1}{3}\sigma_c \varphi \quad . \tag{5}$$

Under an externally applied load, taut actin filaments neither bend nor twist. Instead, they rotate, change spacing, and change their length to attain an equilibrium configuration. The greater the prestress carried by the actin filaments, the smaller the geometrical changes the network must undergo before attaining equilibrium. Consequently, cell rigidity increases nearly proportionally with increasing prestress (Stamenoviæ et al. 1996; Fredberg et al. 1998; Stamenoviæ and Coughlin 1999; Stamenoviæ and Wang 2000).

Several two-dimensional tensed cable networks have been used as models of CSK mechanics (Discher et al. 1998; Coughlin 2000). These models could mimic a number of behaviors observed in cells during mechanical tests and in some cases they also provided good quantitative correspondence to experimental data. However, two-dimensional topology limits the applicability of these models to CSK mechanics of adherent cells in which actin filament reorientation is observed in three-dimensions in response to stress application (Maniotis et al. 1997).

A special case of tensed cable networks is tensegrity architecture (Fig. 4). The distinguishing feature of tensegrity structures is that they are composed of both tensile elements (cables) and compressive elements (struts), which balance tension in the cables. (Tensegrity can be defined as an interaction of a set of discrete compression elements (struts) with a continuous network of tension elements (cables) in the aim of forming a stable form in space (Pugh 1976).)

In adherent cells, actin filaments and intermediate filaments are envisioned as tensile elements whereas microtubules and thick, cross-linked actin bundles as compression elements. In addition to compression elements within the CSK, the ECM also balances a portion of the CSK tension. In other words, CSK and ECM are assumed to form a synergetic, self-equilibrated mechanical system (cf., Ingber 1993). The assumption of existence of compression elements within the CSK has been a point of controversy that has surrounded the cellular tensegrity model for many years (Ingber et al. 2000). Although we have recently shown that microtubules indeed balance a substantial portion of the CSK tension (Stamenoviæ et al. 2002b), we think that this issue of internal versus external balance of CSK tensile stress is of secondary importance (Wang et al. 2002).

During the past six years we intensively studied cellular deformability using tensegrity models (Stamenoviæ et al. 1996; Fredberg et al. 1998; Coughlin and Stamenoviæ 1997, 1998; Stamenoviæ and Coughlin 1999, 2000; Wang and Stamenoviæ 2000; Stamenoviæ and Wang 2000). Recently, other groups have also begun to explore this idea (Wendling et al. 1999; 2000; Volokh et al. 2000). All these studies have shown that tensegrity models can mimic a number of features observed in living adherent cells during mechanical tests including prestress-induced stiffening, strain hardening, and the effect of cell spreading on cell deformability. More importantly, this model helped us to identify the roles and relative contributions of various molecular CSK structures to the observed behaviors and to obtain quantitative predictions. Some of key findings from tensegrity modeling are described below.

According to tensegrity, the role of the actin network is to carry the prestress, thereby conferring shape stability to the entire cell. The role of microtubules is to carry compression as they balance prestress within the actin network. When the compression reaches a critical value, microtubules buckle thereby providing an additional degree of freedom to cell deformability. To evaluate the effect of buckling of microtubules, the post-buckling equilibrium theory has been utilized (Coughlin and Stamenoviæ 1997; Volokh et al. 2000). At large applied strains, intermediate filaments may become dominant tension-bearing components of the CSK whereas

at small strains their contribution is negligible (Maniotis et al. 1997; Wang and Stamenoviæ 2000). Tensegrity models explain the observed strain hardening as a result of geometrical recruitment (reorientation and change in spacing) of CSK components in the direction of applied load. However, under certain loadings, tensegrity models also exhibit softening whereas such behavior has not been observed in cells under similar loading conditions (Coughlin and Stamenoviæ 1997, 1998; Volokh et al. 2000).

Using a six-strut tensegrity model as a representative structural unit of the CSK and subjecting it to uniaxial stretching (Fig. 4), the pulling force versus extension relationship is obtained (Stamenoviæ et al. 1996). To obtain the elastic (Young's) modulus (E), we used an equivalent continuum approximation (see the caption under Fig. 4) and obtained that (Stamenoviæ and Coughlin 1999, 2000):

$$E = 5.85\frac{\sigma_c a}{l^2}\frac{1+4\varepsilon}{1+12\varepsilon} \tag{6}$$

where σ_c is the pre-existing tensile cable stress, l is the corresponding cable length, a is the cable cross-sectional area and ε is the pre-existing cable strain. Taking into account that there are 24 cables in the model and thus their volumetric fraction $\varphi = 24al/V$, where $V = 1.36l^3$ is the volume defined by the model, it follows from Eq. (6) that:

$$E = \frac{\sigma_c \varphi}{3}\frac{1+4\varepsilon}{1+12\varepsilon} \tag{7}$$

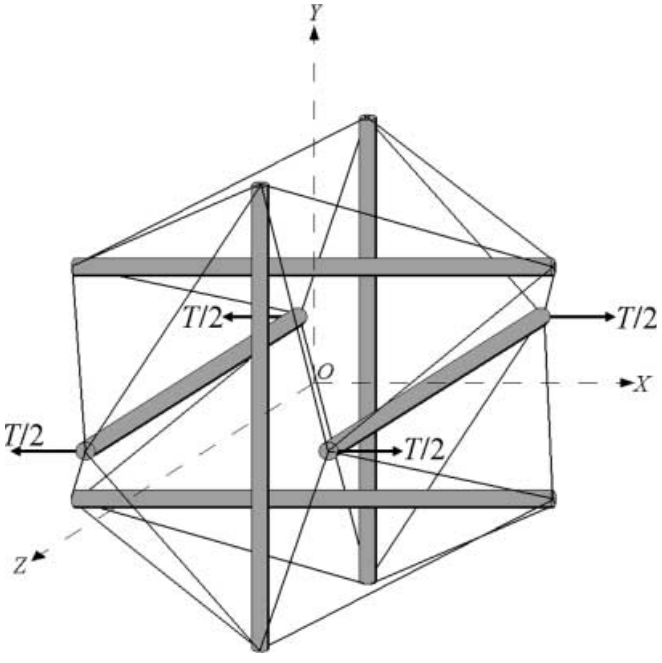

Fig. 4. A six-strut tensegrity model that has been used as a representative unit of the CSK (Stamenoviæ et al. 1996; Coughlin and Stamenoviæ 1997, 1998; Stamenoviæ and Coughlin 1999, 2000; Wendling et al. 1999; Wang and Stamenoviæ 2000; Volokh et al. 2000). Initially, the cables (*thin lines*) carry pre-existing tension that is balanced by compression in the struts (*gray columns*). To calculate stiffness of the structure, a pair of parallel struts was pulled apart by forces $T/2$ applied at each node of the pulled struts and the corresponding displacement (i.e., change of distance between pulled struts) was calculated from governing equations (cf., Stamenoviæ et al. 1996). Stiffness was determined as the derivative of force–displacement relationship at the origin. From this stiffness, Young's modulus (Eq. 6) was calculated using an equivalent continuum approximation as follows (Stamenoviæ and Coughlin 1999). The work of T on the incremental change of distance between pulled parallel struts (δs_x) per reference volume (V) enclosed by the model equals the work of uniaxial stress S_x on an incremental uniaxial strain δe_x, $T\delta s_x/V = S_x\delta e_x$. Here $V = 1.361l^3$ where l is the length of tensed cables before application of T and $\delta e_x = \delta s_x/s_x$ where $s_x = 0.817l$ is the initial distance between parallel struts (i.e., before application of T). The structure Young's modulus $E \equiv \mathrm{d}S_x/\mathrm{d}e_x = (0.5/l)(\mathrm{d}T/\mathrm{d}s_x)$, with the derivatives calculated at the reference (initial) state, where $\mathrm{d}T/\mathrm{d}s_x$ is the structural stiffness, $dT/ds_x = 11.9(\sigma_c a/l)(1+4\varepsilon)/(1+12\varepsilon)$ where σ_c and ε are pre-existing tensile stress and strain in the cable, respectively, of the reference cross-sectional area a (Coughlin 2000)

By substituting Eq. (5) into Eq. (7) it follows that:

$$E = P\frac{1+4\varepsilon}{1+12\varepsilon} \tag{8}$$

where P is the aforementioned prestress. Assuming that the cables are actin filaments, and taking into account that the yield strain of actin filaments is very small (~0.9%) (Tsuda et al. 1996), it follows that $\varepsilon \ll 1$. Moreover, assuming that under a small load, the cell is incompressible and isotropic (i.e., $G = E/3$), it follows from Eq. (8) that the shear modulus is:

$$G \approx \frac{1}{3}P \tag{9}$$

According to Eq. (9), G increases linearly with P, which is consistent with experimental data obtained from cultured airway smooth-muscle cells (Wang et al. 2002). However, the G vs. P slope of ~0.2 obtained from the experimental data is smaller than the slope of 1/3 predicted from Eq. (9). Second, since values of the prestress measured in non-stimulated airway smooth-muscle cells are within $O(10^3)$ (Wang et al. 2002), it follows from Eq. (9) that the predicted value of G falls within the accepted range of measured values of G of $O(10^2$–$10^3)$ Pa.

Taken together, the above results indicate that the tensegrity model provides a good and plausible description of CSK mechanics of adherent cells. The controversy that surrounded this model about the presence of compression-supporting elements within the CSK has been cleared by recent experimental measurements that show, in cultured airway smooth-muscle cells, that microtubules carry a substantial compression as they balance contractile force of the actin network (Stamenoviæ et al. 2002b). However, the fraction of the prestress balanced by microtubules varies. In highly spread cells adherent to elastic gel substrates it can be as low as 13% (Stamenoviæ et al. 2002b), whereas in cells whose spreading was confined and shape predetermined by a micro-patterned substrate it can be as high as 70% (N. Wang, personal communication). Thus, depending on the extent of cell spreading, cell shape, and possibly cell type, microtubules may or may not play a critical role in balancing CSK prestress. This, in turn, suggests that one may not always need to invoke tensegrity to explain mechanical features of the cell. Other types of tensed cable networks or even cortical membrane models may be also appropriate. However, it is important to clarify that cells commonly adhere to relatively flexible substrates within living tissues in vivo and they only rarely exhibit the highly extended forms they exhibit on rigid substrates in vitro. Thus, tensegrity is likely the most relevant model of the cell in its natural physical environment.

4
Alternative approaches

Besides structurally based and mechanistic approaches, there are several other approaches used in studies of mechanics of adherent cells. These include an energetic approach (Stamenoviæ et al. 2002b), percolation and polymer physics theories (Forgacs 1995, MacKintosh et al. 1995; Boey et al. 1998) and a glass-transition model (Fabry et al. 2001). These models are briefly discussed below.

4.1
Energetic approach

We have recently used an energetic approach to calculate contributions of various CSK structures to the overall energy budget of cell during contraction (Stamenoviæ et al. 2002b). The advantage of this approach is that the energy is a scalar quantity, independent of the choice of coordinate system and of the particular details of the CSK architecture. We assumed that the energy of a contracting cell (W) is stored in various structures, including the elastic substrate (W_τ), the tensed actin network (W_{MF}), in buckling microtubules (W_{MT}), and other contributing elements (W_{other}):

$$W = W_\tau + W_{\mathrm{MF}} + W_{\mathrm{MT}} + W_{\mathrm{other}} \ . \tag{10}$$

Assuming that the CSK–ECM system is perfectly elastic (i.e., no energy dissipation), we calculated the componential energies of W based on the elastic and geometrical properties of CSK filaments and on data obtained from prestress measurements of airway smooth-muscle

cells cultured on a flexible gel substrate (Stamenoviæ et al. 2002b). We found that for maximally stimulated (contracted) cells (i.e., W = const.), W_{MF} = 0.02 pJ and W_{MT} = 0.18 pJ; W_{MF} was calculated assuming that the actin filaments are linearly elastic whereas W_{MT} was calculated assuming that microtubules are slender beams laterally supported by intermediate filaments (Brodland and Gordon 1990) and using the post-buckling elastic model (cf., Timoshenko and Gere 1988). Contributions of other relevant CSK structures did not exceed $O(10^{-2})$ pJ. These values were compared with experimental data for W_τ calculated as work done by traction during contraction of airway smooth-muscle cells (Stamenoviæ et al. 2002b). It was found that during maximal stimulation of the cell $W_\tau = 0.64$ pJ, which is nearly two orders of magnitude greater than W_{MF} and by a factor of 3.5 greater than W_{MT}. This, in turn, suggested that most of the contractile energy is stored in the elastic substrate and in buckling microtubules, and much less in the actin network and other CSK structures. Moreover, when we chemically disrupted microtubules in maximally stimulated cells and then measured W_τ, we obtained an increase in W_τ of 0.13 pJ. This increase is attributed to the transfer of energy stored in the microtubules prior to their disruption to the substrate following their disruption. Note that this measured increase in the energy is close to the theoretically estimated value of W_{MT} = 0.18 pJ.

Taken together, results of the energy budget analysis suggest that the role of the actin filaments is to carry the prestress and not to store the contractile energy. The role of microtubules is to balance the contractile prestress. In that process, microtubules buckle, thereby contributing to the cell elastic behavior. The role of intermediate filaments is to stabilize and prevent excessive buckling of microtubules. The contribution of other structures appears not to be significant. These findings are consistent with results from tensed networks models and provide independent evidence in support of the tensegrity cell model.

4.2
Percolation theory and polymer physics models

Forgacs (1995) proposed a model of the CSK based on percolation theory. This model is focused on the connectivity of the CSK as the essential feature that determines the mechanical behavior of adherent cells. In that sense, it is similar to other structurally based models that we examined in this paper. However, the percolation model did not take CSK forces into consideration. Unfortunately, this model did not go beyond its conceptual description and no quantitative predictions of cellular mechanical behavior have been obtained.

Recent approaches have used the physics of CSK filaments in modeling cell mechanics (MacKintosh et al. 1995; Boey et al. 1998). In these approaches, molecular scale phenomena such as thermal motions were considered as essential for describing cell deformability. These phenomena were excluded from the structural models described above. Boey et al. (1998) introduced the thermal motions into the structurally based cable network model of the erythrocyte cortical CSK. It was shown in a companion study that this model could successfully predict mechanical behavior of erythrocytes during micropipette aspiration measurements (Discher et al. 1998). To obtain an optimal correspondence to experimental data, the model needed to be prestressed. This suggests that forces associated with thermal motions of the molecules of the CSK are not sufficient to explain fully the overall cell behavior and that the central mechanism of prestress is needed for a complete description of CSK mechanics.

4.3
Glass-transition model

Most of the structurally based models considered above (open-cell foams and stress-supported structures) are static and deterministic. As such, they are not suitable to describe dynamic properties of the cell. Impedance (oscillatory) measurements on smooth-muscle cells as well as other cell types reveal that cell elastic (storage) and frictional (loss) moduli increase with frequency according to a weak power low (Fabry et al. 2001), and that at a given frequency these moduli increase linearly with increasing CSK prestress (Stamenoviæ et al. 2002a). Whereas stress-supported structures in which elastic structural members are replaced by simple viscoelastic ones can account for the observed dependences of elastic and frictional moduli on the prestress (Stamenoviæ et al. 2002a), they cannot describe the observed power-low frequency dependence of these moduli (Fabry et al. 2001). This type of behavior implies a wide spectrum of viscoelastic time constants, whereas stress-supported models with simple viscoelastic members that are characterized by a discrete number of time constants can mimic a power-low behavior only over a limited range of frequencies (C. Sultan, personal communication). One may argue that a stress-supported structure whose individual members follow

a power-low behavior would be able to mimic the observed behavior of cells, or that a multimodular structure composed of many simple stress-supported units, each of which is characterized by a different time constant, may also create the power-low effect. However, there is no firm rational basis for such a design. Instead, Fabry et al. (2001) proposed an alternative mechanism, a glass-transition model, which is fundamentally different from all types of models considered above.

It has been suggested that common rheological features (i.e., the weak power-low behavior) of materials that exhibit glass transition reflect generic system properties at some higher level of structural organization rather than particular molecular properties (Sollich 1998). Unlike the deterministic models where structural elements are always in equilibrium and assume geometry that minimizes their energy state, in the glass-transition model, structural organization is at a metastable, non-equilibrium state. The dynamic modulus (mechanical impedance) for a material that exhibits power-low behavior is given as follows (Fabry et al. 2001):

$$G^* = G_0\left(\frac{\omega}{\Omega_0}\right)^{x-1}(1+i\eta)\Gamma(2-x)\cos\frac{\pi(x-1)}{2} \tag{11}$$

where G_0 and Ω_0 are scaling factors for stiffness and frequency, respectively, ω is radian frequency, (x–1) is the power law exponent, $\eta \equiv \tan[\pi(x-1)/2]$, $\Gamma(\cdot)$ is the gamma function and i is the imaginary unit indicating the out-of-phase behavior. The real part of Eq. (11) is the elastic modulus and the imaginary part is the frictional modulus. The range of behaviors described by this theory extends from a perfectly elastic solid (glass transition) of stiffness G_0 to a viscous liquid, depending on the effective noise temperature x. As x increases, the material behavior transforms from glass ($x = 1$) towards liquid ($x = 2$). In this case, Ω_0 is the frequency at which the system departs from the stable glass transition. Fabry and colleagues (2001) suggested that CSK proteins might regulate cell mechanical properties mainly by modulating the effective noise temperature of the CSK network. The implication of this is that the effective noise temperature is a measure of the CSK ability to deform and flow. It is not yet known, however, how the effective noise temperature can be regulated in the cell (by drugs, mechanical stimuli, etc.), or how it may depend on how far is the cell from thermodynamic equilibrium. Furthermore, at its current state, this theory cannot account for the effect of prestress that appears to be a key determinant of both static and dynamic behavior of adherent cells.

It is noteworthy that the power-low behavior is not uncommon in biorheology. It characterizes behavior of various soft tissues. In the past this power-low behavior was explained by a reptation model (Suki et al. 1994) or a 1/f noise model (Bates et al. 1994). These models could also explain the observed cell behavior.

5 Conclusion

In this survey we have evaluated various types of models of CSK mechanics. We concluded that structurally based models, designed according to the rules of stress-supported structures, appear to be most appropriate to describe the central role of the CSK prestress in determining mechanical behavior of adherent cells at the steady state. Which subclass of stress-supported structure may be appropriate for describing cell deformability type (e.g., cortical membrane model or tensegrity model) depends on the extent of cell spreading, cell type, and level of question (e.g., molecular versus cellular) being addressed. These deterministic models may not be well suited to describe the dynamic behavior of cells characterized by a weak power-low frequency dependence of their elastic and frictional moduli. In this case, non-equilibrium rheological theories, such as the glass-transition model, which focus on a generic system property rather than a single molecular mechanism, may be more appropriate. However, to have value for biology, all of these models must provide some molecular or structural correspondence within living cells.

We should point out that we omitted a number of chemically-mediated mechanisms that are widely believed to influence cell mechanical behavior (cf., Amos and Amos 1991). These include CSK remodeling, acto-myosin molecular motor kinetics, phosphorylation, cross linking, etc. Although the effects of these mechanisms on CSK mechanics are well documented, each of them cannot describe various facets of cell mechanical behavior as in the case of the central role of the CSK prestress. We believe that the mechanisms described in structurally based models as well as in the non-structurally based complex models, such as the

glass-transition model, elucidate a higher level of organization in which biochemical modulating events may function and be regulated.

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