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Review

Rheological behavior of mammalian cells

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Abstract. Rheological properties of living cells determine how cells interact with their mechanical microenvironment and influence their physiological functions. Numerous experimental studies have show that mechanical contractile stress borne by the cytoskeleton and weak power-law viscoelasticity are governing principles of cell rheology, and that the controlling physics is at the level of integrative cytoskeletal lattice properties. Based on these observations, two concepts have emerged as leading models of cytoskeletal mechanics. One is the tensegrity model, which explains the role of the contractile stress in cytoskeletal mechanics, and the other is the soft glass rheology model, which explains the weak power-law viscoelasticity of cells. While these two models are conceptually disparate, the phenomena that they describe are often closely associated in living cells for reasons that are largely unknown. In this review, we discuss current understanding of cell rheology by emphasizing the underlying biophysical mechanism and critically evaluating the existing rheological models.

Keywords. Cytoskeleton, prestress, power law, tensegrity, soft glass rheology, actin networks, viscoelasticity, contractility.

Introduction

The ability of mammalian cells to deform in response to mechanical forces is essential for many integrated cellular functions, including spreading, contraction, migration, secretion, apoptosis and mechanotransduction [1–5]. Deformability of cells is governed by mechanical properties of the cytoskeleton (CSK) that determines stability of the cell's shape and actively generates contractile forces [6–10]. Mechanical properties of the CSK arise from the complex network of biopolymers that comprise the cytoskeletal lattice, which undergoes continuous remodeling and is driven by specialized molecular motors that convert chemical energy of adenosine triphosphate (ATP) into mechanical forces. A major challenge in cell mechanics is to identify physical laws that govern deformability, contractility and remodeling of the CSK and, based on these laws, to develop quantitative models that link cell's structure to its function.

A growing body of experimental evidence has shown that mechanical behaviors of adherent cells are governed by two major principles: (i) the CSK exists in the state of tension (also called "prestress") that is critical for stabilizing cell shape and for regulating cell rigidity; and (ii) cytoskeletal rheological behaviors are driven by a very slow dynamics such that global viscoelastic responses of cells scale with time and frequency of loading according to a weak power law. Various models have been proposed to describe different aspects of these phenomena [11–24], but two models have emerged as the most robust and unifying concepts – the tensegrity model [9] and the soft glass rheology (SGR) model [25]. The tensegrity model, which depicts the CSK as a prestressed, self-equilibrated, stable network of opposed tension-

supporting and compression-supporting elements, can predict how cell stiffness increases with increasing level of the prestress (*i.e.*, stiffening behavior), but it does not naturally predict the power-law rheology. The SGR model, which depicts the CSK as a non-equilibrium, metastable system, predicts the power-law rheology, but cannot account for the dependence of those behaviors on the prestress. While these two models are conceptually very different, experimental data have shown that in living cells the power-law viscoelasticity is closely associated with the cytoskeletal prestress, indicating that these two phenomena may be linked together.

In this article, we discuss phenomena that characterize rheological behaviors of living adherent cells. We focus on the cytoskeletal prestress and the power-law viscoelasticity as principal determinants of cell rheological behaviors, discuss the underlying mechanisms and critically evaluate current models of cell rheology. We end the discussion by briefly describing the challenge for future investigations in this area.

Molecules of the cytoskeleton

The CSK is composed of three major filamentous biopolymers: actin microfilaments, microtubules, and intermediate filaments, and a number of cross-linking proteins (cf. [26]). In general, cytoskeletal biopolymers are much less flexible than synthetic polymers, yet they can still exhibit significant bending fluctuations driven by thermal Brownian motion and thereby influence the soft viscoelastic response of the CSK. In polymer physics a measure of filament flexibility is given by the persistence length (L_p), which is roughly the minimum length at which the filament ends become uncorrelated to Brownian motion [27]. If the filament length is shorter than its L_p, then the filament behaves as a straight elastic rod. If the filament length is longer than L_p, then the filament appears wavy, which is indicative of thermal bending fluctuations. Filamentous actin (F-actin) has L_p on the order of 10^1 μm and a high Young's elastic modulus (E ~10^3 MPa), which is a measure of the filament tensile stiffness [28]. Tensile tests of isolated actin filaments show that their tension-extension relationship measured up to maximum physiological tension (~230 pN) is largely linear, except at low tensions (0–50 pN) where it exhibits a nonlinear 'toe' region that reflects a decrease in entropy due to conformational changes as the filaments become fully stretched [29]. However, within the CSK of living cells, actin filaments are much shorter (<1 μm) than their L_p and thus they appear as straight line segments [30]. This, in turn, suggests that conformational (entropic) changes of individual actin filaments have little contribution to viscoelasticity of cells, and that their contribution is mainly through their participation in the deformable cytoskeletal network. Cytoskeletal actin filaments are often cross-linked with myosin motor proteins that are capable of generating tensile force in the actin filaments through the ATP-driven process of cross-bridge cycling. As a result of this action, the CSK becomes prestressed. Cytoskeletal actin filaments are also found grouped together with myosin and other actin-binding proteins to form bundles known as actin stress fibers. Tensile tests on isolated actin stress fibers show that they are much less stiff (E ~10^0 MPa) and much more extensible than individual actin filaments, and that they exhibit a marked nonlinearly elastic behavior characterized by stiffening [31]. This stiffening behavior may contribute to the overall elastic response of the CSK.

Isolated microtubules appear as straight rigid tubes and have nearly the same elastic modulus as actin filaments (E ~10^3 MPa), but much greater L_p ~10^3 μm [28]. Therefore, one would expect microtubules to appear straight on the whole cell level as well. However, immunofluorescence images of CSK-based microtubules show that they appear bent and wavy [9, 32, 33]. It follows, therefore, that some type of internal mechanical force must act on microtubules; conceivably the bent and wavy shapes of CSK-based microtubules indicate their buckling under compression as they oppose contractile stress of the actin network [32–34].

Intermediate filaments are much more flexible (L_p ~10^0 μm) and much less stiff (E ~10^0–10^1 MPa) than either actin filaments or microtubules. Like stress fibers, they are highly extensible and exhibit stiffening behavior [35]. In living cells, a typical length of intermediate filaments (10–20 μm) is much greater than their L_p, which explains their wavy appearance [4]. This suggests that conformational changes of intermediate filaments contribute to the viscoelastic response of cells [35]. However, this contribution appears to be minor; they only start to significantly contribute to the overall mechanical response of the cell during large cell deformation, when intermediate filaments become fully extended and stretched [36–39]. Another important mechanical role of intermediate filaments is to provide a lateral elastic support to microtubules, like lateral guide wires that stabilize compression-bearing microtubules against buckling [40].

Although all three major filamentous biopolymers are important for mechanical functions of the cell, their relative contributions differ. Experimental studies in which these biopolymers were selectively disrupted show that the contractile actin has a major contribu-

tion to the overall mechanical response, whereas the contributions of microtubules and intermediate filaments are relatively smaller [7, 41, 42].

Mechanical properties of individual cytoskeletal filaments provide useful information about their mechanical roles in the CSK. However, these properties cannot provide quantitative information about global mechanical properties of the CSK. Biomechanical measurements on living cells have shown that cytoskeletal stiffness is of the order of 0.1–1 kPa [25, 32, 43–47], which is six to seven orders of magnitude lower than the tensile stiffness of individual actin filaments and microtubules. A simple way to reconcile this huge disparity between the filament stiffness and the cytoskeletal network stiffness is to use an affine assumption [18, 22]. Based on this assumption, one can show that the global network stiffness is determined by elastic modulus (E) of individual network elements and by their volumetric fraction (ϕ) in the network. If the main mode of deformation of individual elements is tension, then the network stiffness (or the effective elastic modulus) is directly proportional to $E\phi$. If the main mode of element deformation is bending, then the network stiffness is directly proportional to $E\phi^2$ (cf. [48]). Note that it is not thermally driven bending, rather it is mechanically driven by externally applied forces. Taking into account that for actin filaments $E \sim 10^9$ Pa and that their volumetric fraction in living cells is $\phi \sim 10^{-3}$, the affine tensional filament model would predict the cytoskeletal stiffness of $\sim 10^3$ kPa, whereas the affine bending filament model would predict the cytoskeletal stiffness of ~1 kPa [18, 22]. Clearly, the former is a high overestimate of the global cytoskeletal stiffness, whereas the latter represents its upper bound. Moreover, measured stiffness of purified actin gels does not exceed $\sim 10^{-1}$ kPa [49]. Since in living cells filaments undergo both bending and tension, it follows from the above analysis that the cytoskeletal stiffness ranges between ~1 and $\sim 10^3$ kPa, which is much higher than the values obtained from experimental measurements. Thus, a more detailed description of cytoskeletal microstructure and of mechanical interaction between cytoskeletal components is needed to obtain accurate predictions of the cytoskeletal stiffness. One such possible description is given by the cellular tensegrity model, which we discuss in the following section.

Control of cytoskeletal mechanics by prestress

Critical to cell shape stabilization is the fact that the actin network of the CSK carries mechanical tensile stress (prestress), even before application of an external force, and this prestress is transmitted *via* the cytoskeletal lattice to all of the structural elements of the cell. Based on these observations relating to the mechanical stability and connectivity of the CSK, Ingber [9, 50–52] proposed a model that describes the CSK as tensegrity architecture, a building system that utilizes mechanical balance between tension and compression elements to create a self-equilibrated stable mechanical structure (Fig. 1A). The pivotal idea of the cellular tensegrity model is that cytoskeletal prestress is generated through establishment of a complementary force balance between contractile microfilaments that actively generate tensional forces and other intracellular and extracellular molecular structures that oppose and balance these forces. Experimental data from various studies confirm that cells utilize this type of force balance to self-organize and to stabilize their CSK [7, 4, 32, 34, 38, 39, 53–56] and to determine and tune their cytoskeletal stiffness [32, 42, 43, 57–59].

Cytoskeletal contractile stress is transmitted to the extracellular matrix (ECM) *via* integrin focal adhesion (FA) plaques [60]. Traction forces that arise at the extracellular adhesions are largely responsible for opposing cytoskeletal contractile forces [32, 43, 61]. However, contractile forces are also opposed by cytoskeletal microtubules (Fig. 1B) that can buckle in that process, but carry high levels of compressive forces ($\sim 10^2$ pN) per microtubule when laterally supported by intermediate filaments [32, 34] or by viscous cytoplasm [33]. This complementary force balance at an FA can be described by a simple relationship: $\mathbf{T} = \mathbf{F}_{MF} - \mathbf{F}_{MT}$, where $\mathbf{T}$ is the traction force vector, $\mathbf{F}_{MF}$ is the tensile force vector of actin microfilaments and $\mathbf{F}_{MT}$ is the compression force vector of microtubules (Fig. 1B).

The tensegrity force balance provides a way to shift forces between various load-bearing molecular elements in the cell, and thereby has a direct impact on their self-assembly behavior through altering their chemical potential. In general, tensile forces tend to lower the chemical potential of a molecular aggregate relative to the molecular reservoir (cytoplasm) and thus promote molecular assembly, whereas compressive forces tend to increase the chemical potential of the aggregate and thus promote its disassembly (cf. [62, 63]). For example, if actin tension, $\mathbf{F}_{MF}$, is generated by activation of contractile motors then, based on the relationship from Figure 1B, both compression of microtubules, $\mathbf{F}_{MT}$, and traction, $\mathbf{T}$, on FAs will increase, providing there are no major geometrical changes at the FA level. Consequently, actin filaments and FAs will assemble [64–69], whereas microtubules will buckle and disassemble, as observed in living cells [70]. On the other hand, if

$\mathbf{F}_{MF}$ increases due to passive mechanical straining of the ECM then, based on the complementary force balance from Figure 1B, **T** also will increase while $\mathbf{F}_{MT}$ will diminish. Consequently, actin filaments, FAs and microtubules will assemble, and this is again what is observed in living cells [54, 68, 71–74].

The cellular tensegrity theory has proven to be robust and predict multiple mechanical behaviors of various cell types from first mechanical principles [22, 39, 75–88]. Thus, given the relevance of tensegrity for biomechanics of living cells, it may be helpful to provide a more detailed description of this building system.

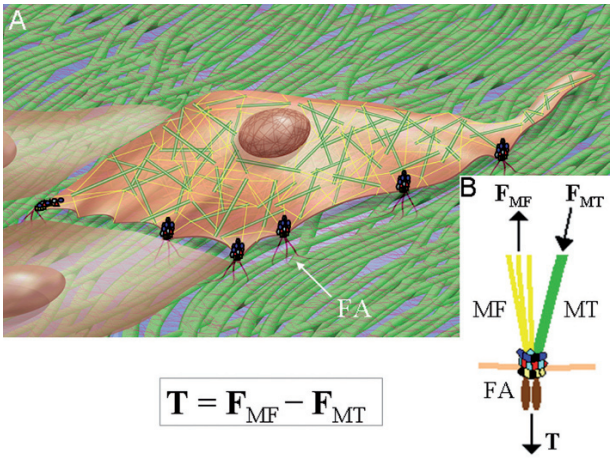

Figure 1. (*A*) An artistic depiction of the cellular tensegrity model. The cytoskeleton is comprised of a continuous network of tensile actin filaments (yellow lines) and isolated microtubule struts (green). The cytoskeleton is linked to the extracellular matrix *via* focal adhesion (FA) molecular clusters. Designed by artist Matt Pickett and Donald E. Ingber; courtesy of Donald E. Ingber. (*B*) A schematic representation of the complementary force balance between tension ($\mathbf{F}_{MF}$) in actin microfilaments (MF), compression ($\mathbf{F}_{MT}$) of microtubules (MT) and traction forces (**T**) at the focal adhesion (FA) contacts.

Tensegrity structures can be viewed as an interaction set of a continuous network of tensile elements with discontinuous (isolated) compression elements that create stable forms in space [89, 90]. The tensile elements carry pre-existing tension (*i.e.*, prestress), which confers stability to the structure; the compression elements counterbalance the tension. Together, they form a self-equilibrated stable mechanical system, whereas in the absence of prestress, these structures collapse. A typical example of a tensegrity structure is a circus tent where tension in the cloth and cables is opposed by compression in the poles and by the ground pegs to which the cables are attached. The greater the tension in the cloth and cables, the more stable (*i.e.*, more rigid) the whole structure will be. In the absence of tension (e.g., if the cables are cut or disconnected from the pegs) the tent would collapse. The reason that tensegrity structures tend to collapse in the absence of the prestress is that rigidity and connectedness of their structural elements are insufficient to fully constrain their freedoms of motion and thus stabilize the structure. Consequently, tensegrities are not intrinsically stable structures (like, for example, rubber or metals are), and thus they require the prestress for stabilization. When an external force is applied to a tensegrity structure, it deforms such that its structural elements undergo geometrical rearrangements (primarily rotation and change in spacing) until a new equilibrium configuration is attained. The greater the prestress, the smaller the deformation the structure has to undergo before attaining equilibrium, *i.e.*, the stiffer it is. This explains the stiffening behavior of tensegrities with an increasing level of prestress, which is characterized by a linear relationship between stiffness and prestress. Importantly, the manner in which prestress is generated and balanced does not qualitatively affect this relationship. This prestress could be generated actively, as in a contractile CSK, or passively by mechanical distension of the network (*e.g.*, by mechanical stretching of the substrate to which the cell adheres). It also may be balanced internally by compression elements (*e.g.*, microtubules) and/or by attachments to external objects (*e.g.*, adhesions to the ECM and to neighboring cells).

The central mechanical stabilizing role of prestress is fundamental to all published cellular tensegrity models, regardless of whether highly simple or more complex [22, 39, 75–88], and this is the key reason why these models yield results that are highly consistent with observations in living cells.

Results from biomechanical measurements on various types of cultured adherent cells indicate that changes in cytoskeletal prestress are paralleled by changes in cell stiffness, regardless of the means by which the prestress is modulated or the types of cells or experimental techniques that are utilized. For example, treating airway smooth muscle cells with contractile (*e.g.*, histamine, bradykinin, serotonin, endothelin-1, KCl), and relaxing (*e.g.*, isoproterenol, DBcAMP, forskolin, ML-7) agents causes an increase and a decrease in cell stiffness, respectively [25, 32, 42–44, 47, 58, 59, 91–94] in a dose-dependent fashion [32, 42, 43, 58, 59, 91]. An increase in cell mechanical distension by either uniform (*i.e.*, equibiaxial) stretching of the substrate [57, 95–97], by varying cell spreading in a controlled manner [53], or by osmotic swelling of the cell [98], results in an increase in cell stiffness. Importantly, studies in which cell stiffness and cytoskeletal prestress are measured independently show that there is a linear relationship between

the stiffness and the prestress (Fig. 2) [32, 43, 59], which is also predicted *a priori* by a cellular tensegrity model [22, 59, 81, 85]. In those measurements, cell stiffness was measured by applying local stress to the CSK *via* integrin receptors, while the contractile prestress was measured on the whole cell level. Different metrics of the contractile prestress were used in those measurements, some dependent on cell size and shape and some that are not. In all those cases, a linear association between cell stiffness and contractile stress was obtained, suggesting that it is independent of details of cell geometry [43].

There are alternative interpretations of the observed stiffening behavior of cells. Cell treatments with contractile agonists cause an increase in the number of myosin crossbridges attached to actin filaments and also induces polymerization of cytoskeletal F-actin. Both effects are known to cause an increase in cell stiffness [99–102]. Thus, the observed stiffening behavior in response to contractile agonist treatments may be nothing more than the effect of cytoskeletal remodeling and/or myosin crossbridge recruitment. However, increasing the passive component of the prestress by rapid biaxial stretching of the substrate, which has very little effect on myosin crossbridge recruitment or on actin polymerization, also produces cell stiffening [57, 95–97]. Furthermore, even when myosin crossbridge recruitment is inhibited (by DBcAMP), cells still exhibit stiffening in response to substrate stretching [97].

Another interpretation of the prestress-induced stiffening is that it reflects elastic nonlinearity of individual cytoskeletal components (*i.e.*, a phenomenon known as stress hardening), for example due to nonlinear bending of cytoskeletal filaments [103]. Using actin networks as an *in vitro* model of the CSK, it has been shown that a network composed of filaments shortened by gelsolin to their physiological length (*i.e.*, lengths much shorter than F-actin L_p) and cross-linked with filamin-A exhibits stiffening with increasing prestress (Fig. 3) [49, 104]. This stiffening behavior is attributed to elastic nonlinearity of filamin-A. However, in living cells filamin-A appears to have a minor effect on cellular mechanical responses [105]. In another study, cross-linked actin gels with dispersed myosin motors were activated *via* ATP [106]. The gels stiffened with increased levels of activation-induced tension in actin filaments, similarly to living cells. Importantly, in an earlier study where non-cross-linked actin gels were activated by ATP, they exhibited fluidization, not stiffening [107]. The reason is that activation does not induce tension in entangled actin filaments, rather it enhances their longitudinal motion, which results in gel fluidization on the macroscale. Taken together, these results demonstrate the importance of cross-linking (*i.e.*, connectivity) of the cytoskeletal lattice for buildup and transmission of prestress, and ultimately for cytoskeletal rigidity.

While interpretations of cell stiffening behavior *via* mechanisms other than tensegrity cannot be completely ruled out, the fact that this behavior persists, regardless of how the prestress is modulated, of cell types and of experimental techniques utilized, strongly suggests that the stiffening is governed by tensegrity mechanisms.

There have been some prominent critics of the cellular tensegrity idea, mainly centered on the role of microtubules as compression-supporting elements of the CSK [108, 109] and the static elastic nature of cellular tensegrity models [110]. The controversy surrounding the role of microtubules has been alleviated and refuted by experimental findings showing that in living cells microtubules carry a substantial compressive load [32–34, 111]. Because of the existence of the complementary force balance (see Fig. 1B), the relative contribution of compression of microtubules *versus* ECM traction to the cellular force balance changes with cell spreading. With increasing cell-ECM contact formation, the contribution of traction forces that oppose cytoskeletal contractile forces increases at the expense of compression of microtubules. In fact, experimental data confirm that in highly spread cells, microtubules balance a small percentage of the contractile prestress, whereas in poorly spread cells microtubules balance nearly 50 % of the prestress [111]. Since in their natural habitat cells seldom exhibit highly spread forms, their contribution to balancing the prestress cannot be ignored.

The CSK is a non-elastic and dynamic remodeling network, exposed to dynamic external loads, which challenges adequacy of static elastic tensegrity models in cytoskeletal mechanics. For example, it has been shown that in response to transient uniform substrate stretching (a brief stretch-unstretch maneuver), adhering airway smooth muscle cells initially soften ("fluidize") and then solidify [112]. The greater the prestress before the cells are subjected to transient stretching, the greater the extent of this fluidization. These observations ostensibly conflict with the idea that an increase in mechanical distension of cells leads to buildup of cytoskeletal prestress, which in turn leads to cell stiffening through tensegrity mechanisms. Trepat and co-workers [112] argued that this fluidization is consistent with the behavior of soft glasses, which are dynamic, metastable, non-equilibrium materials (see next section for details), quite opposite from static, self-equilibrated, stable tensegrities. Another possibility is that transient stretching produces structural disorder within the CSK such that the

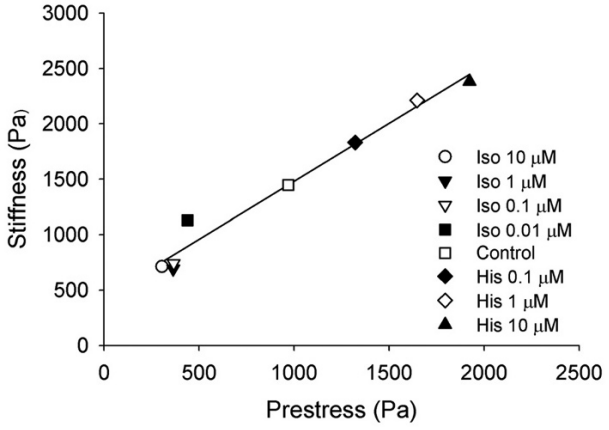

Figure 2. Cell stiffness increases linearly with increasing contractile prestress. Measurements were carried out in cultured airway smooth muscle cells whose contractility was modulated by graded doses of contractile agent histamine (His) and a relaxant agent isoproterenol (Iso). The stiffness was measured using the magnetic cytometry technique [92] and the prestress was measured using the traction cytometry technique [43]. Data are means ± SE; solid line is a linear regression. (Adapted from Wang et al. [43].)

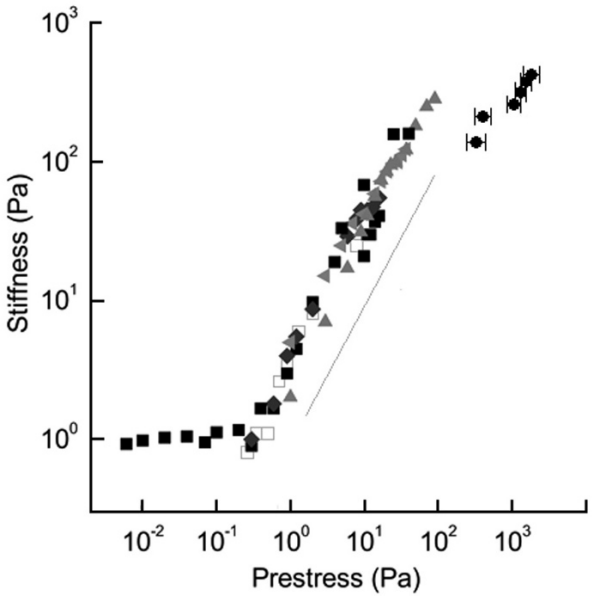

Figure 3. Stiffness of actin networks cross-linked with filamin-A increases with increasing prestress. Measurements were carried out using a stress-controlled parallel plate rheometer in gels with different actin concentration (c_A) and different molar ratios of filamin A (R): c_A =36 μM, R =1/100 (open squares); c_A =48 μM, R =1/100 (solid squares); c_A = 4 μM, R =1/100 (diamonds); c_A =36 μM, R =1/50 (left-pointing triangles); c_A =53 μM, R =1/50 (upward-pointing triangles). For comparison, data from measurements in living airway smooth muscle cells (Fig. 2) are included (solid circles near the top right corner). (From Gardel et al. [49].)

complementary force balance between molecular components is temporarily lost, which leads to a loss of structural stability and to fluidization. Over a longer timescale, however, the structural organization of the CSK and force balance is regained through structural remodeling, which leads to the observed solidifying of the CSK through restoration of prestress. However, these purely mechanical explanations ignore the interrelation between mechanics and molecular assembly. For example, this type of rapid stretching also induces a rapid increase in intracellular cAMP and calcium levels [113] that can promote a wave of cytoskeletal depolymerization followed by repolymerization, which could explain these results.

In summary, the overwhelming body of evidence indicates that the cytoskeletal prestress is a key determinant of cell stiffness and stiffening behavior, and that this is consistent with the cellular tensegrity model. While this description well characterizes the steady-state behavior where cytoskeletal elastic forces predominantly contribute to the overall cell stress response, under dynamic loadings viscous forces also come into play. Could the prestress influence cytoskeletal viscous stresses as it does elastic stresses? This question is addressed in the next section.

Power-law rheology of cells

In response to applied dynamic and static forces, cells continuously deform such that their viscoelastic material moduli scale with frequency (f) and time (t) of the imposed deformation according to a weak power law, $\sim f^{\alpha}$ and $\sim t^{\alpha}$, where $0.05 \leq \alpha \leq 0.35$ (Fig. 4). The scaling exponent α can be viewed as an index of transition between Hookean elastic solid-like ($\alpha = 0$) and Newtonian viscous fluid-like ($\alpha = 1$) behaviors [25, 44, 57]. This power-law behavior extends over a wide range of frequencies (10^{-2}–10^{3} Hz) and persists over a wide range of experimental conditions, regardless whether cells are probed locally or on the whole cell level [25, 44–48, 92–95, 114–118]. At high frequencies (10^{2}–10^{3} Hz), the weak power law crosses over to a more dependent power-law regime with α approaching the value of 0.75 (see Fig. 4) [94, 118], which is indicative of entropic dynamics of semiflexible actin [18, 20, 119]. However, these high frequencies are not physiologically relevant and thus we focus only on the power-law regime below 10^{3} Hz. Fabry and colleagues [25] recognized that the weak power-law behavior conforms to an empirical law known as structural (hysteretic) damping, which suggests that the phase lag between viscous and elastic stresses that develop within a material body is independent of the frequency of loading. While the physical basis for the power-law behavior of living cells remains unclear, these data rule out simple models with a discrete number of time constants (*e.g.*, the Kelvin-Voigt model, the Maxwell model, and the

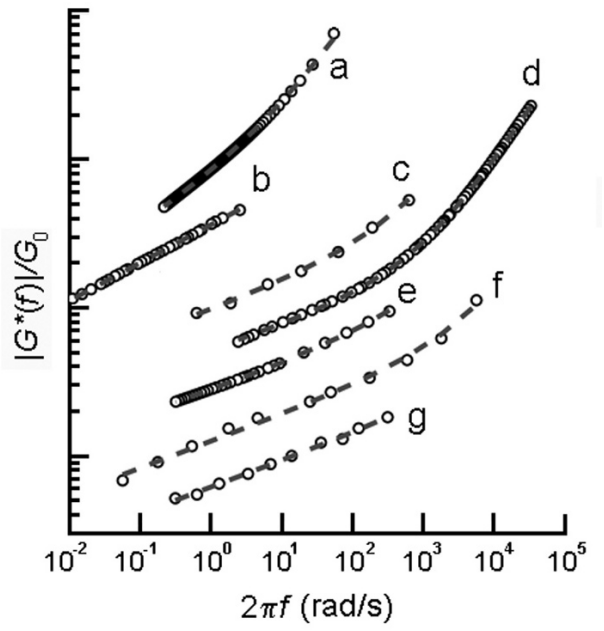

Figure 4. Power-law frequency responses of different cell types measured by different techniques: (a) endothelial cells, magnetic pulling [115]; (b) myoblasts, uniaxial rheometry [116]; (c) epithelial cells, atomic force microscopy [45]; (d) COS-7 cells, laser tracking microrheology [114]; (e) airway smooth muscle cells, magnetic bead twisting [93]; (f) airway smooth muscle cells, oscillatory magnetic cytometry [25]; and (g) myoblasts, optical tweezers [46]. $|G^*|$ is the magnitude of the dynamic modulus, G_0 is a scaling factor for stiffness, and f is forcing frequency. The log-log slope of the curves is indicative of the scaling power-law exponent α. At lower frequencies, α ranges from 0.18 to 0.26, whereas it increases at higher frequencies and approaches the value of 0.75, indicative of the entropic dynamics of cytoskeletal actin. Points are data and dashed lines are best fits based on the power-law function $Af^{\alpha} + Bf^{0.75}$ where A and B are constants. (Adapted from Hoffman et al. [118].)

standard linear solid), although models composed of a large number of the discrete units with distributed time constants (*e.g.*, generalized Kelvin-Voigt and Maxwell models) can simulate the power-law behavior [120]. On the other hand, the weak power law characterizes viscoelastic responses of inert soft materials, which belong to a class of materials known as soft glasses.

During recent years, rheology of soft glasses (SGR) – a semi-empirical theory derived from soft matter physics [121] – has gained a great interest in cell biology since this theory can account for various dynamic behaviors observed in living cells. Because soft glasses include a diverse type of materials, including foams, emulsions, slurries, pastes and colloid suspensions, their common mechanical behavior must not be determined by specific molecular mechanisms and organization since they differ between materials. Instead, their common mechanical behavior reflects generic system properties at some higher level of structural organization. These generic features are that their ultrastructure is discrete, disordered, crowded, metastable and away from thermodynamic equilibrium. Due to the crowdedness, structural elements (whatever they may be) are trapped by interactions with neighboring elements (*e.g.*, cross-links, hydrophilic interactions, charge effects, or steric constraints). To escape these traps, thermal Brownian fluctuations alone are not sufficient. Instead, elements are envisioned as being agitated and jostled by their mutual interactions with neighboring elements. Physical origins of these non-thermal agitations remain unknown; however, their influence can be represented by an empirical, non-dimensional parameter referred to as an effective noise temperature (x); when $x = 1$, particle cannot escape from their energy traps and material behavior is perfectly elastic; this state is known as the glass transition; when $x > 1$, the elements can escape from the traps and the microstructure is becoming disordered. As a result, the material can flow like a fluid. Under applied mechanical stress, soft glasses undergo structural rearrangements. In that process, structural elements cross over non-thermal energy barriers. On the macroscale, this results in a very slow, flow-like deformation of a soft glass system such that its material moduli scale with time and frequency of loading as weak power law. According to the SGR theory, the power-law exponent is directly related to the effective noise temperature, *i.e.*, $\alpha = x - 1$ [121].

To assess microstructural dynamics of the CSK, spontaneous motions of intracellular [122] and cell surface bound tracer particles have been measured [123, 124]. These particle motions are believed to represent cytoskeletal remodeling and contractile dynamics [121, 122]. It has been shown that spontaneous motions of Arg-Gly-Asp (RGD)-coated beads bound to integrin surface receptors (which are physically linked to the CSK) exhibit patterns that are characterized by periods of confinement (in energy traps) punctuated by hopping events (jostled out of energy traps) [123, 124], which are strikingly similar to the theoretical description of microscale dynamics of soft glasses [121]. These measurements also show that particle fluctuations are non-thermal. By measuring the mean square displacement (MSD) of particle tracers, it can be shown that it increases with time as a power law, $\text{MSD} \propto t^{\beta}$ [122–124]. If the cytoskeletal microstructure were in thermodynamic equilibrium, where microstructural dynamics is thermally driven, then, according to the generalized Stokes-Einstein relationship [125], the exponent of bead motion β has to be equal to the rheological scaling exponent α and therefore β should increase in a direct proportion as α increases. In contrast, experimental data show that β decreases with increasing α (Fig. 5), indicating a breakdown of the generalized Stokes-

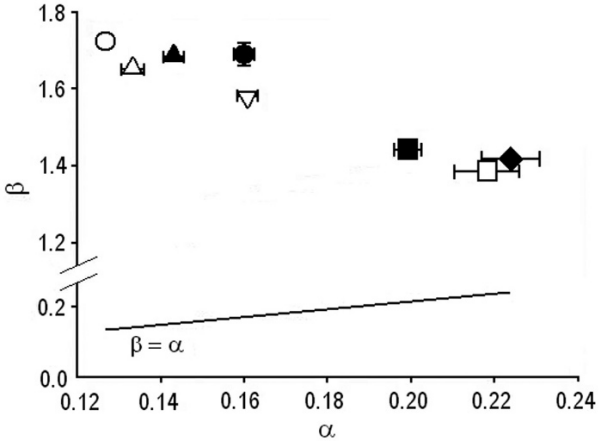

Figure 5. Exponent β obtained from the time course measurements of the mean square displacement of spontaneous motions of a bead marker attached to an integrin receptor on the surface of airway smooth muscle cell *versus* the power-law exponent α obtained from rheological measurements on the same cell using magnetic twisting cytometry. Data are means ± SE obtain for the following interventions: actin stabilizer Jasplakinolide (○; 1 μM), contractile agonist histamine (Δ; 0.1 mM), 23°C (▲), ATP depleted (●; 2 mM deoxyglucose + 2 mM NaN_3), 37°C (▽), relaxant agonist DBcAMP (■; 1 mM), 41°C (□), and actin disruptor cytochalasin D (◆; 2 μM). The solid line β = α represents the prediction from the dissipation-fluctuation theorem. (Adapted from Bursac et al. [123].)

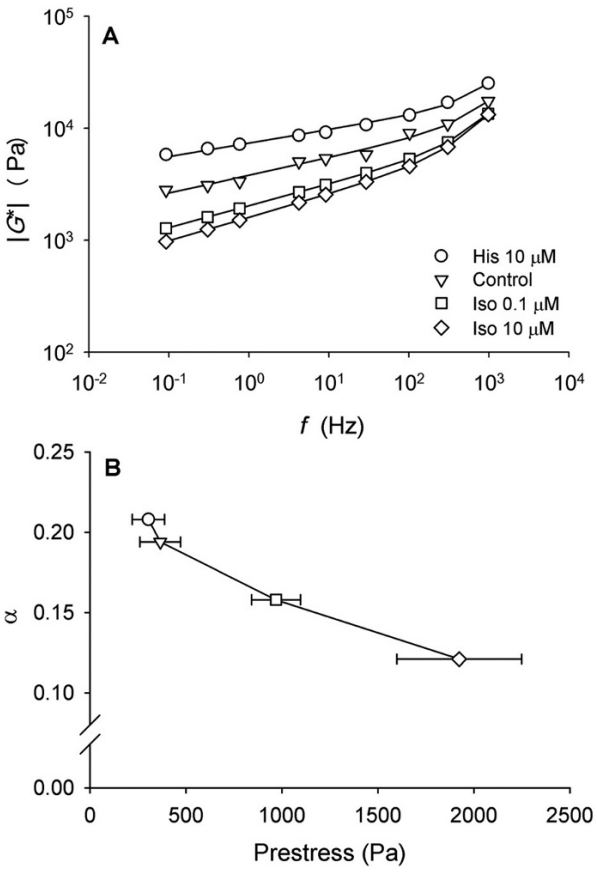

Figure 6. (*A*) Magnitude of the dynamic modulus (|*G**|) of cultured airway smooth muscle cells increases with increasing frequency of loading (*f*) following a weak power law. At higher frequencies (>100 Hz), the power-law dependence increases and approaches the log-log slope of 0.75, presumably due to increasing contribution of entropic dynamics of cytoskeletal actin. Measurements were carried out using the magnetic twisting cytometry technique under control conditions and following treatments with a contractile agonist histamine (His) and a relaxant isoproterenol (Iso). Data are means, SE do not exceed 5 % and are not shown; solid lines are best fits based on the power-law function $Af^{\alpha} + Bf^{0.75}$ where *A* and *B* are constants. (*B*) Exponent α decreases with increasing contractile prestress. Data for α are means ± SE obtained from the log-log slopes of the power-law relationships in Figure 6A, and data for the prestress are means ± SE obtained from measurements in Figure 2. (Adapted from Stamenović et al. [58].)

Einstein relationship and thereby departure of the system from thermodynamic equilibrium [123]. It was also found that MSD increases when cells are treated with agents that lower cytoskeletal prestress (myosin light chain kinase inhibitor ML-7, Rho-kinase inhibitor Y-27632) and *vice versa*, MSD is reduced when cells are treated with agents that increase the contractile prestress (platelet-derived growth factor, arsenite) [124, 126]. Interestingly, since MSD is inversely related to stiffness [122, 123, 125] while, according to the tensegrity model, the stiffness increases with increasing prestress, the above results suggest that the prestress regulates both stiffness and glass dynamics of the CSK. The SGR model can explain the power-law rheology of cells; however, it cannot account for its dependence on the cytoskeletal prestress. It has been shown that during oscillatory measurements on living cells, the magnitude of the dynamic modulus (|*G**|, defined as the ratio of the applied stress amplitude to the corresponding strain amplitude) follows a weak power law, ~f^{α}. By increasing the prestress, |*G**| also increases, whereas α decreases (Fig. 6). These prestress-dependent relationships do not depend on whether the prestress is modulated *via* stimulating/relaxing myosin contractile motors [25, 44, 58, 93, 94] or passively, by mechanical stretching of the substrate [95, 96]. Since α is an index of transition between elastic solid and viscous fluid behaviors, it follows that this transition is controlled by the cytoskeletal prestress. On the other hand, since SGR implies that $\alpha = x - 1$, it again follows that the prestress controls cytoskeletal dynamics, *i.e.*, that increasing prestress reduces the level of the effective noise temperature *x*.

In addition to the power-law rheology, soft glassy materials are characterized by aging, rejuvenation and fluidization behaviors, which have also been observed in living cells [112, 122]. It has been shown that cells stiffness increases as the time interval between two subsequent stress applications increases – a process known as aging. This process can be reversed following a short application of a large amplitude oscillatory stress after which the cell recovers its original stiffness

– a process known as rejuvenation [122]. It has also been shown that, in response to transient substrate stretching, cells exhibit rapid softening – a process known as fluidization – followed by a slow regaining of stiffness [112]. Importantly, all these soft glassy behaviors of living cells are shown to be dependent on the level of cytoskeletal prestress.

The above results show that changes in glass-like activities and changes in the state of contractility are closely associated in living cells. This lead to an interpretation that the non-thermal fluctuations of the cytoskeletal microstructure are ATP-related and driven by contractile forces [110, 112, 122, 124]. Much is known about regulation of actin-myosin contractile force *via* activation of the small GTPase Rho, which in turn acts through its downstream effect Rho-associated kinase (ROCK) to enhance myosin light chain phosphorylation and at the same time promotes actin polymerization through another effector, mDia [66]. However, a physical mechanism that links cytoskeletal contractile forces and cytoskeletal glass dynamics to one another is not known. There are alternative explanations for the power-law rheology of living cells. The transition between the fluid and solid behavior has been often framed in the context of sol-gel transition [14, 127, 128]. Gels near a critical gelation point exhibit power-law phenomena (cf. [129, 130]) similar to soft glasses near glass transition [121]. In that regard, it is important to point out that both gel transition and glass transition are believed to arise from the same mechanisms, *i.e.*, kinetic arrest due to crowding of clusters [131]. The power-law behavior of living cells could also result from folding and unfolding and from conformational changes of cytoskeletal proteins [132, 133].

One strategy in the investigation of cell rheology is to use purified actin networks as a minimal *in vitro* model of the CSK. This approach avoids the complexity of measurements in living cells and instead focuses on few mechanisms that are believed to be critical determinants of mechanical properties of the CSK. While earlier investigations with entangled actin networks enjoyed only a modest success in describing power-law rheology and elasticity of cells [19, 36, 119, 134, 135], recent investigations that were carried out with cross-linked actin networks showed more promises. Rheological studies on actin networks with shortened and cross-linked (filamin-A) filaments, exhibit a cell-like weak power-law behavior ($\alpha = 0.1$) over the same frequencies range as living cells [136]. The power-law exponent α also shows a similar dependence on the prestress as living cells do (see Fig. 6B). These cell-like behaviors, as well as the prestress-dependent stiffening (see Fig. 3) of the cross-linked actin networks, suggest that they may have captured the governing mechanisms of cytoskeletal mechanics. Although those mechanisms are still not completely understood, they certainly do not include ATP-dependant contractile activities, which have been indicated as a driving force of the cytoskeletal dynamics in the SGR model. Therefore, the dependence of the power-law exponent on prestress that is observed in both cells and cross-linked gels seems to be a generic property of viscoelastic prestressed structures rather than a specific ATP-dependent activity.

Interestingly, tensegrities also can explain the observed dependence of $|G^*|$ on the prestress if elastic tensile elements in a tensegrity structure are replaced by viscoelastic Voigt elements (*i.e.*, a spring and a dashpot in parallel) [82, 83, 87]. During deformation of such a tensegrity structure, its viscoelastic elements undergo geometrical rearrangements, which cause a redistribution of their viscoelastic time constants such that a qualitatively new viscoelastic behavior of the structure as a whole emerges. Since geometrical rearrangements in tensegrities are governed by the prestress, the time constant redistribution becomes prestress dependent, which explains why $|G^*|$ changes with the prestress. Since tensegrity models are composed of a discrete number of structural elements, with a discrete number of time constants, they cannot predict the power-law rheology, which implies a continuous spectrum of time constants. However, it has been shown that with a suitable distribution of discrete time constants even a simple tensegrity model can mimic the power-law behavior of cells over a given range of loading frequencies [83]. Given the hierarchical nature of living tensegrity systems that have structural elements of various size scales [9], these elements may naturally display a large range of time scales that could contribute to this rheological response.

A single power-law regime implies that macroscopic deformation of a material body is governed by processes that are time-scale invariant, regardless of the physical basis for this behavior [25, 44, 93]. Recent measurements of the oscillatory response of airway smooth muscle cells over an extended range of frequencies (10^{-3}–10^{3} Hz), which includes physiological frequencies, indicate the existence of two distinct power-law regimes in the $|G^*|$ *versus* f relationship separated by a plateau (Fig. 7) [137]. Similar behavior was also reported for other cell types, under different loading conditions [116, 117, 137, 138]. Importantly, the transition between the two the power-law regimes takes place within the physiological range of frequencies, indicating a biological significance of this type of behavior. Two power-law regimes separated by a plateau region over a well-defined timescale imply

that rheological processes are not timescale invariant. Mechanisms that lead to this behavior in living cells remain largely unknown. A theoretical model of myosin-activated entangled actin networks can predict a similar behavior [139] but, as we already mentioned, entangled actin networks are not adequate model for describing other aspects of cytoskeletal mechanics. While the SGR model allows the possibility that at very low frequencies a power-law regime changes to another regime [121], its current mathematical formulation predicts only a single power-law regime and thus a timescale invariant behavior [25, 44, 93]. Whether the SGR model can be modified to account for the observed two power-law regimes remains to be seen.

In summary, the weak power-law, aging, rejuvenation, and fluidization behaviors and the breakdown of the generalized Stokes-Einstein relationship that are observed in living cells are signatures of glassy dynamics, which have made the SGR model very attractive as a paradigm of cell rheology. However, these phenomena are also closely associated with the cytoskeletal prestress in living cells – a behavior that the current SGR model cannot explain. Cross-linked actin networks, on the other hand, can replicate the basic power-law and prestress-dependent behaviors of living cells, suggesting that the mechanisms that they embody may be essential for understanding cytoskeletal mechanics.

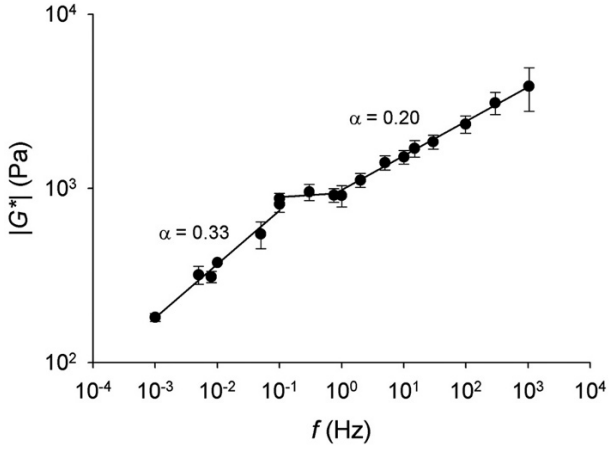

Figure 7. The dynamic modulus ($|G^*|$) *versus* frequency (f) relationship measured in cultured human airway smooth muscle cells using the magnetic twisting cytometry technique [25]. Cells displayed two power-law regimes at 10^0–10^3 Hz and at 10^{-3}–10^{-1} Hz, separated by a plateau (10^{-1}–10^0 Hz). Each regime was fitted by a function $\sim f^{\alpha}$ (solid lines). In the frequency range 10^0–10^3 Hz, the power-law exponent $\alpha = 0.2$, whereas in the frequency range10^{-3}–10^{-1} Hz, $\alpha = 0.33$. Data are means ± SE. (Adapted from Stamenović et al. [137].)

Can tensegrity and SGR models be linked together?

It is well known that some types of inert soft glassy materials, such as liquid foams and emulsions, have prestress-dependent stiffness [140, 141]. This property of foams and emulsions is commonly framed in the context of tensegrity mechanics [142, 143], showing that tensegrity principles are not alien to soft glassy materials. However, while relatively simple microarchitecture and micromechanics of foams and emulsions makes it easy to understand how these materials exhibit both tensegrity-like [142, 143] and SGR-like behaviors [144, 145], it is much less obvious in the case of the complex, dynamic and contractile cytoskeletal material. A cue that may link these two types of behaviors in living cells may come from the observation that, while the prestress stabilizes the cytoskeletal lattice *via* tensegrity mechanisms, it also reduces the effective noise temperature and brings the cell closer to the glass transition behavior, although the mechanisms for the latter are unknown. Thus, rather than viewing the tensegrity and SGR models as two mutually incompatible concepts of cytoskeletal mechanics, it is more appropriate to regard them as two complementary models that within their own rights can describe a broad class of phenomena observed in living cells, and which are linked to one another through prestress.

Two new models were recently proposed with the aim of linking the power-law rheology to prestress [146, 147]. Both models focus on the dynamics of individual semiflexible polymers under tension. A key premise in those models is that mechanical tension carried by semiflexible polymers of a prestressed cytoskeletal lattice influences their molecular dynamics and thereby affects the power-law rheology of the whole CSK. Both studies used the worm-like chain (WLC), which is a minimal model of a semiflexible polymer [27], as a basis of their modeling. One of those models considered a discrete version of the WLC, known as the elastic-jointed chain, which describes the polymer as a chain comprised of nonlinearly elastic segments connected by linear torsional springs [146]. Assuming that chain's dynamics is thermally driven, the model predicts a weak power-law viscoelastic response such that the power-law exponent decreases with increasing level of tension, consistent with the observed behavior of living cells (Fig. 6B). The nonlinear elasticity of the chain is a key factor that links the power-law to the prestress. However, the assumed thermally driven chain dynamics is inconsistent with the observations that cytoskeletal dynamics is non-thermally driven (see Fig. 5). This problem is alleviated in the second model, which describes the viscoelastic response of a glassy (*i.e.*, non-thermally

driven) chain under tension. This is accomplished by modifying (exponential stretch) the relaxation time spectrum of the standard WLC [147, 148]. In this way, the model effectively links together SGR and prestress. While the model predictions compare favorably with experimental data from purified actin gels, they enjoy less success when compared to the experimental data from living cells [148].

Summary

Numerous experimental studies have shown that cytoskeletal contractile prestress and power-law viscoelasticity are governing principles of the rheology of adherent cells, and that the controlling physics is at the level of integrative cytoskeletal lattice properties. Together, these two principles define the most fundamental properties of the cytoskeletal phenotype, the ability to deform, contract and remodel, which are critical for higher cellular physiological functions, including motility, mitosis, apoptosis, differentiation, and mechanotransduction. The theoretical challenge for bioengineers and biophysicists for the future is three-pronged. First, to make the cellular tensegrity model useful for describing dynamic behaviors of living cells, current static tensegrity models of the CSK must be replaced by a new generation of dynamic tensegrity models. This not only means replacing passive elastic tensegrity elements with viscoelastic ones, but, more importantly, incorporating contractile force generation and remodeling dynamics into tensegrities. Second, if the SGR model is to become a unifying model of cytoskeletal mechanics, it has to be able to account for the prestress-dependent behaviors of cells. Identifying molecular mechanisms that link the effective noise temperature to the cytoskeletal prestress could be a step in that direction. Third, recent studies have shown that cross-linked actin gels have a potential to explain both power-law and prestress-dependent rheological behaviors observed in living cells. Thus, future studies of cross-linked actin gels could lead to an *in vitro* model of the CSK that can replicate the essential rheological behaviors of living cells. If any of these tasks could be accomplished successfully, it may become possible to obtain a unifying, physically based structure-function model of cytoskeletal mechanics. Such a unifying description, amenable to computational and structural analysis in the context of the cytoskeletal molecular structure, would be a useful tool for cell biologists and other life scientists for studying and understanding a broad range of cellular functions.

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