

Isostaticity and Controlled Force Transmission in the Cytoskeleton: A Model Awaiting Experimental Evidence

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ABSTRACT A new model is proposed for force transmission through the cytoskeleton (CSK). A general discussion is first presented on the physical principles that underlie the modeling of this phenomenon. Some fundamental problems of conventional models—continuous and discrete—are examined. It is argued that mediation of focused forces is essential for good control over intracellular mechanical signals. The difficulties of conventional continuous models in describing such mediation are traced to a fundamental assumption rather than to their being continuous. Relevant advantages and disadvantages of continuous and discrete modeling are discussed. It is concluded that favoring discrete models is based on two misconceptions, which are clarified. The model proposed here is based on the idea that focused propagation of mechanical stimuli in frameworks over large distances (compared to the mesh size) can only occur when considerable regions of the CSK are isostatic. The concept of isostaticity is explained and a recently developed continuous isostaticity theory is briefly reviewed. The model enjoys several advantages: it leads to good control over force mediation; it explains nonuniform stresses and action at a distance; it is continuous, making it possible to model force propagation over long distances; and it enables prediction of individual force paths. To be isostatic, or nearly so, CSK networks must possess specific structural characteristics, and these are quantified. Finally, several experimental observations are interpreted using the new model and implications are discussed. It is also suggested that this approach may give insight into the dynamics of reorganization of the CSK. Many of the results are amenable to experimental measurements, providing a testing ground for the proposed picture, and generic experiments are suggested.

INTRODUCTION

Many cell functions, including growth, motility, gene expression, apoptosis, and signal transduction, are controlled by mechanical stresses and alterations in cell shape (1–3). Forces are transmitted between the interior of cells and extracellular matrix through transmembrane receptors, the most relevant of which for this discussion are probably integrins (4). Within the cell, stresses are mediated to a large extent by the cytoskeleton (CSK), a network of actin filaments (AFs), intermediate filaments (IFs), and microtubules (MTs). This filamentous structure extends throughout the cytoplasm. The receptors connect to the CSK at focal adhesion points, effectively linking the nucleus to the surface of the cell.

Conventional models treat the cell as a membrane enclosing a continuous internal medium that may be elastic, viscous fluid, or viscoelastic (5–10), but this approach has been questioned in recent years. There is evidence that such descriptions of the cell do not capture key features of the mechanics of cells, such as the nonuniformity of strain distribution inside the cell (6,11–13) and directed propagation of force stimuli over large distances across CSK networks (14,15). These phenomena cannot be accounted for by conventional continuous descriptions without resorting to complex anisotropic constitutive properties correlated over large distances across the cell. This led to suggestions that

the intracellular mechanics may be better understood if the effects of the discreteness of the CSK were taken explicitly into consideration (12,13,16–20).

The short- and long-time responses of cells to forces are governed by different mechanisms and therefore require different modeling. A force stimulus applied to the cell first propagates away from the stimulation point through the mechanical response in the medium due to minute changes in internal strains. These changes take place on timescales that are very short compared to biochemical responses, which propagate only at the speed of diffusion, $\text{distance} \sim \sqrt{\text{time}}$. Although it is clear that biochemical activity plays an important role in intracellular mechanotransduction, driving the dynamics of reorganization of the CSK, the difference in timescales relegates it to trail the initial mechanical response. In fact, the latter triggers all this subsequent activity. Therefore, for a complete description over a wide range of timescales it is essential to first understand the basic mechanics. A comprehensive theory must include the combined effects of both mechanisms and the interplay between them, but the construction of such a theory is beyond the scope of this article. Rather, I concentrate here on the development of a fundamental model for control through mechanical response at short timescales. The hope is that such a model will serve as a stepping stone to more complex versions that would include the mechanochemical aspects. Possible implications of the model for the biochemical reorganization of the CSK will be discussed briefly in the concluding section.

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Of particular interest are observations of “action at a distance”, namely that upon pulling a bead attached to a receptor in the membrane, the relatively distant nucleus changes shape (14,15,18,19). This type of response suggests that localized force stimulation of membrane receptors can be communicated reliably to the vicinity of the nucleus without grossly distorting the entire cell.

Although the implication of the discreteness of the CSK structure in occurrence of nonuniform stresses appears to make sense on some conceptual level, it introduces several difficulties. One is that stress propagation through discrete networks is sensitive to the structural details of the network and therefore quantitative predictions of discrete models would depend strongly on model details. Another difficulty, hardly addressed in the literature, is that nonuniform force transmission and action at a distance requires particular network structural characteristics, as will be discussed in detail below. A third problem concerns the methodology of science in general. A model should provide accurate predictions, for example, on the details of the intracellular response to force stimuli at the membrane. However, to compute the forces at every structural element of the CSK is a hopeless task: the CSK network not only contains many filamentous elements, but its structure also changes constantly, both from cell to cell and in time. The default description of many-element systems is normally by continuum coarse-grained models, and blaming the continuous nature of conventional stress models for the failure to account for the observed phenomena leaves one with a difficult decision regarding the introduction of an alternative. These problems have severely hindered the development of a first-principles predictive model of force mediation in the CSK.

It is in this context that one should view the controversy that arose after the proposal of Ingber and co-workers that the CSK may be described as a tensegrity structure (21,22). The term tensegrity, short for tensional integrity, had been coined by R. Buckminster Fuller in the 1920s, and it refers to stable structures of force-carrying stiff rods and taut strings (23). Although Ingber’s idea dates back to the 1980s, its usefulness has remained controversial. One critique of it is that even on the static level it is difficult to translate this idea into detailed predictions on intracellular propagation of stresses. Another problem is quite fundamental: tensegrity cannot really explain nonuniform stresses on scales much larger than the mesh size of a framework, an issue that will be discussed in more detail below. Consequently, tensegrity toy models (16,17,25–27) are too small to be useful on a quantitative level, and predictions made on the basis of descriptions of the CSK as ordered lattice networks (28) are not only unrealistic, but also miss a key point, as will be made clear below.

The discrete picture has been taken to an extreme by Wang and Suo (20), who, following observations of forces guided by particular actin bundles in human airway smooth muscle cells, analyzed only the one-dimensional behavior along such bundles. The main problem with this approach is that it ignores the three-dimensionality of the CSK, artificially

decoupling the high-dimensional contractile forces that lead to prestress (see discussion in the concluding section) from the force stimulus that is mediated into the cell. For example, that model cannot predict the path that the force will take into the cell, nor how mediated stimuli would superpose on the prestress.

The claim of this article is that the observations of action at a distance have implications that go well beyond the idea of tensegrity and may call for a shift of paradigm altogether. The central aim of this article is the resolution of the above difficulties and the development of a generic model for the transmission of force stimuli in the CSK. The structure of this article is as follows. First, a general discussion is presented on the issue of cell control over mediation of forces from a designer’s point of view. This discussion elucidates several advantages of transmission of channeled forces through the CSK. Next, the requirements of the model to be developed are specified. These provide clear criteria against which models in general, and this one in particular, must be tested. The performance of conventional continuous models is then examined against the requirements. Two fundamental premises that appear to underlie the recent drift in preference toward discrete models are identified as misconceptions and put straight.

This preliminary discussion forms the basis for the main proposal of this article, that a natural way to achieve the functionality requirements and to explain the observations of nonuniform stresses and action at a distance is for large regions of the CSK to be isostatic or near-isostatic. Isostatic states are then defined, and a brief review is presented of isostaticity theory. Explicit solutions for stresses in two-dimensional isostatic systems are presented, demonstrating that forces can be communicated controllably from the boundary into the cell. Next, a quantitative analysis is developed of the structural conditions that the CSK must satisfy to be isostatic. Due to lack of data, several possible scenarios are analyzed. This analysis makes it possible to test the isostaticity idea by direct observations of particular statistical properties of the CSK, and potential experimental tests for doing so are suggested. The results are summarized in the concluding section and some implications of the model for the reorganization dynamics of the CSK are suggested.

Control over stress transmission

Cells constantly need to respond to mechanical stimuli applied at receptors on the membrane. In many cases, the response involves adjustment of the internal structure, where the nature of the adjustment strongly affects the functionality of the cell. Consequently, shape changes must be controlled to a very good precision. Shape changes are directly linked to the way that intracellular stresses are mediated and the understanding of the control mechanisms that govern mechanical stress transmission in the cell has been one of the central quests in the field.

Suppose one is required to design such a mechanism from fresh. Efficiency requires that several basic principles must guide such a design: 1), minimal complexity; 2), a response time that is short relative to other processes in the cell; 3), the force communicated to the nucleus should relay as much information as possible about the position and nature of a faraway stimulation (e.g., at the membrane); and 4), the information should be communicated with as little attenuation as possible.

A transmission mechanism based on an initial mechanical response satisfies both the first and second requirements; it is simpler to manage than the coordination of an array of biochemical reactions and it takes place over timescales that are much shorter than chemical reactions, which rely, among other factors, on diffusion.

To satisfy the third requirement, it should be possible to determine in the neighborhood of the nucleus both where force stimuli originate at the membrane and the characteristics of the stimulating force. The simplest way to achieve this is by retaining *directional* information; extending a line in the direction that the force comes from gives information about the rough location of the stimulus at the membrane.

The fourth requirement is essential, because the multitude of functionalities that take place simultaneously in cells give rise to “noisy” environments. A signal transmitted from the membrane has to travel a long way through such an environment and still be detected above the noise. This places restrictions both on the largest possible rate of attenuation of the signal and on its magnitude. One way to maintain a large magnitude is by keeping the transmitted forces focused as they propagate through the CSK (see Fig. 1).

Having specified the requirements of the system, let us now consider what would be regarded as a good model. First and foremost, it should give the best possible combination of specifications 1–4, which should allow a good level of precision in the communication of forces across the CSK. Second, the model should be able to explain experimental observations, in particular, nonuniform distribution of

stresses in the cell and action at a distance. Third, it should go beyond qualitative explanations and conceptual understanding and provide quantitative predictions against which it could be tested. Ideally, such predictions should include forecast of the spatial distribution of forces that develop in the CSK. This means that, given a force stimulus at a particular location at the membrane (say, point A in Fig. 1), the model should be able to predict, at least in principle, whether the force is felt at point B and its magnitude at that point. As will be discussed in the concluding section, this requirement is also important in understanding the effects of the static picture on the dynamic reorganization of the CSK.

The fourth requirement touches at the heart of the ongoing controversy: should the model be discrete or continuous? This issue is not straightforward and requires some discussion. Continuous models are the default description of most physical phenomena. This is despite the fact that, at some basic level, whether molecular, particulate, or celestial, all systems are made of discrete elements. The main reasons for this are: practicality—one is often interested in behavior on lengthscales (and, correspondingly, timescales) that are much larger than the discrete elements and therefore the wealth of data on the discrete level is often more prohibitive than it is helpful—and convenience—analysis of continuous functions is easier for theorists than the manipulation of discrete functions. Thus, whenever possible, the first attempt at modeling any physical system of many ingredients has always been on the continuous level. But this should not be done at all cost. For example, in a network it would be useless to describe continuously phenomena that take place on the scale of the discrete mesh size. A continuous model is a coarse-grained description that is useful only when the measurable, or interesting, phenomena are on lengthscales much larger than this size. Thus, to construct a continuous model it is essential to first understand the dominant mechanisms on the discrete level and then build a continuous description that is a faithful representation of those mechanisms.

Vis-à-vis the requirements listed above, conventional continuum models of stress transmission in the CSK do not seem to perform well. The main argument against them is that they give rise to *uniform* propagation of stresses from the point of force stimulus (e.g., point A in Fig. 1 b). This both disagrees with observations and dissonates with requirements 3 and 4. To construct a better model, one has to first analyze the reason for this shortcoming. The following argument demonstrates that the problem is quite fundamental and it lies in the premises that are built into the equations of conventional models. Specifically, it is the premise that continuous-stress equations must involve information on deformations. Such information could be either in the form of stress-strain relations, in the case of static models, or in the form of stress-strain rate relations, in the case of viscoelastic models. This idea is paradigmatic in current modeling of

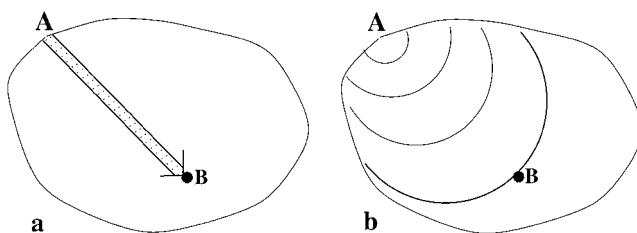


FIGURE 1 Localized force stimulus is applied at point A and is felt at point B through the cytoskeleton that extends between these points. Two modes of propagation of the force are sketched: (a) directed propagation, where the force is mediated through a narrow channel; and (b) dispersive propagation, where the stress becomes more uniform as the distance from A increases. In b, the force felt at point B is a small fraction of the original stimulus, because the stimulus force is supported practically by a large surface, e.g., the thick arc in the sketch.

mechanical stresses and it can be traced back to Saint-Venant (29), who introduced compatibility conditions to calculate stresses in elastic solids. Since elasticity theory is the flagship of this paradigm, it is convenient to illustrate the problem within this context and in two dimensions.

In mechanical equilibrium the continuous stress field must satisfy force and torque balance conditions, which in two dimensions can be written in the form

$$\sigma_{xy} = \sigma_{yx} \text{ (torque balance)} \quad (1)$$

$$\frac{\partial \sigma_{xx}}{\partial x} + \frac{\partial \sigma_{xy}}{\partial y} = g_x \text{ (force balance in } x); \quad (2)$$

$$\frac{\partial \sigma_{xy}}{\partial x} + \frac{\partial \sigma_{yy}}{\partial y} = g_y \text{ (force balance in } y). \quad (3)$$

In these equations, σ_{ij} are the components of the stress tensor ($i, j = x, y$) and $\vec{g}(\vec{r})$ is an external force field applied to the medium. Body forces can be disregarded in the following analysis without loss of generality. The three equations 1–3 are not enough to solve for the four components of the stress tensor σ_{ij} and Saint-Venant proposed to close the set of equations in two steps. First, impose a compatibility condition that the material remain continuous under deformation. In two dimensions this provides one additional equation that relates the second derivatives of the strain field. Second, introduce a further *independent* constitutive relation between the stress and the strain. The same procedure works in three dimensions. There are nine components of the stress tensor to determine, but only six balance conditions—three on forces and three on torque moments. The equations required to close the set are provided by imposing compatibility conditions, which interrelate the second derivatives of the strain field, and then supplementing these conditions with stress-strain relations.

The involvement of the gradients of the strain field has a crucial consequence: it makes the stress field equations *elliptic*. It is because of this very feature that the stress field becomes increasingly uniform away from a localized force stimulus, as sketched in Fig. 1 *b*. This type of solution not only disagrees with observations of action at a distance, but also clashes with requirements 3 and 4 in that they attenuate along any path from *A* to *B* and make it difficult to identify at *B* the origin of the force stimulus. Thus, it is the involvement of compatibility conditions that undermines the use of continuous elasticity for the description of intracellular stresses. The above argument can be readily extended to viscoelastic models since those use compatibility and strain-rate information to close the stress equations. Not surprisingly, viscoelastic models are also hard pressed to explain highly nonuniform stress propagation.

Thus, we arrive at a significant conclusion: the attenuation of stresses in conventional continuous models stems from a basic principle built into the equations, *not from the description of the stress as a continuous field*. It should be emphasized that not all continuous models lead automatically to

dispersion of stress. For example, *hyperbolic* equations give rise to solutions that propagate nonuniformly along characteristic lines. It follows that continuous models may not be as inadequate as believed, and their rejection merely for being continuous may be misguided.

Let us consider now the other end of the argument, the seeming advantages of discrete models. Other than the discontent with continuous models, the main drive behind the move in this direction was the seemingly intuitive idea that discrete structures make it possible to explain both the observations of nonuniform intracellular strains and action at a distance. This idea is based on a presumption that, simply by being discrete, structures can retain directional information and guide forces in a focused manner. This, however, is a misconception! In most known discrete structures, focusing of forces can only take place on the scale of the mesh size; over larger distances, this is usually no longer the case. After all, all materials are made of discrete elements at some basic scale, yet many materials display dispersion of stresses on macroscopic scales as if they are elastic or viscoelastic. The action at a distance is often observed over distances that are considerably larger than the mesh size of the CSK. This calls to question the idea that the mere introduction of discreteness can guarantee directional force mediation over scales comparable to the size of the CSK.

In view of these conceptual problems and the aforementioned advantages of continuous modeling, it seems that the loss of faith in the potential of the latter for stress transmission in the CSK may be premature.

To summarize, any good model of intracellular stress transmission should satisfy well defined requirements and, although conventional continuous models fall short, current discrete models are based on a misconception and continuous modeling in general need not be precluded.

However, to be useful, a continuous model must give rise to a set of nonelliptic equations, and the question is whether a physically plausible such description can be constructed. Ideally, we should start from a discrete picture that supports action at a distance and coarse-grain it consistently to the continuum while retaining this feature. The key to a successful such approach is the observation that discrete high-dimensional networks transmit forces over distances in a focused manner if they are in *isostatic states*. The definition of these states and the way that stress is transmitted in isostatic media are discussed next.

Isostatic states and isostaticity theory

It is convenient to describe isostaticity in one class of isostatic structures, statically determinate systems. A stable structure of discrete elements is statically determinate when all the forces that its components exert on one another can be determined from statics alone. Equations of statics involve only balance conditions, of forces and of torque moments. A high-school textbook example of such a structure is a ladder

of a given weight standing on a rough floor and leaning against a smooth wall (see Fig. 2). The floor exerts on the ladder forces in the x and y directions, whereas the wall exerts on the ladder only a force in the x direction. In mechanical equilibrium, the three forces can be determined by three balance conditions on the ladder, two of force and one of torque moment. A point to note is that the determination of the forces requires no information whatever about the constitutive properties of the material that makes the ladder, e.g., its Young's modulus.

The same is true for systems of many "ladderlike" elements: static determinacy is independent of the number of elements, which can be as large as 10^{12} in some materials. The observation that the forces can be determined regardless of the compliance of the structural elements has a striking consequence. Balance conditions contain information only about spatial distribution of forces, whereas compliance is information that relates the forces to deformations. This means that in statically determinate structures the forces are independent of any deformation-based information. Now, the stress field is only a continuous representation of the field of discrete forces. It follows that information about strains or strain rates is redundant for the determination of stresses in such media. Since strain-related information is at the foundation of elasticity and viscoelasticity, then the field equations of these conventional theories are inadequate to describe isostatic states. This already gives a first indication that isostatic networks may not transmit stresses like conventional materials.

Whether a framework is in an isostatic state depends on its structural characteristics and the type of forces that it can carry. To identify the relevant structural properties that give rise to such states, it is useful to analyze a simple example

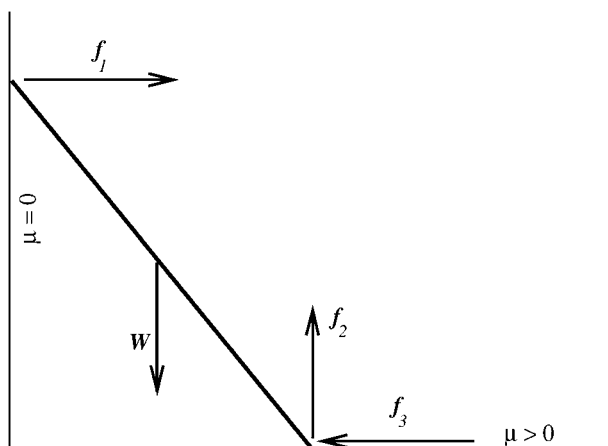


FIGURE 2 Ladder of weight W standing on a rough floor is leaning against a smooth wall. The friction coefficient of the floor is $\mu > 0$, whereas that of the wall is $\mu = 0$. The floor and the wall react to the forces that the ladder applies on them via the forces \vec{f}_1 , \vec{f}_2 and \vec{f}_3 . In mechanical equilibrium, the three forces can be determined by balance conditions alone—of forces and of torque moments. The forces are independent of the compliance of the material of the ladder.

first. Consider a framework of many thin struts connected rigidly at arbitrarily positioned junctions. It is well known from Maxwell's work (30,31) that for such structures to be statically determinate the mean number of elements connecting at the junctions, \bar{z} (coordination number, degree, or valency), has a particular value, z_c (32) when averaged over all junctions. (The analysis presented here presumes for simplicity that the number of junctions is very large, which means that the ratio of boundary to bulk junctions is very small. It is possible to show that this assumption can be lifted and therefore that the argument holds for any system of finite size (R. Blumenfeld, unpublished data).) When this condition is met the number of force components in the struts is equal to the number of balance equations that one can construct for the junctions. Frameworks with $\bar{z} < z_c$ are mechanically unstable and would rearrange spontaneously under the influence of an external load. In frameworks where $\bar{z} > z_c$, not all the forces can be determined from the balance equations and additional conditions are required. Consequently, the global stress transmission in the latter structures is not perfectly isostatic, but may resemble in some regions the behavior of conventional solids (R. Blumenfeld, unpublished data).

The understanding of stress transmission in isostatic media has improved considerably in recent years, particularly in the context of granular assemblies and cellular solids (34–36). Of particular significance to this discussion is a recent development of isostaticity theory for cellular solids (36,37). The theory starts from the discrete Newton's equations for isostatic structures and provides a *continuous* description of the equations that govern the coarse-grained stress field. A detailed description of the theory can be found in references (36–38). In the following, only the salient points for two-dimensional systems are reviewed.

The closure equation of isostaticity theory in two dimensions comes from a local torque-balance condition. This condition relates between the local stress and a specific fabric tensor p_{ij}^v , which characterizes the local details of the structure (see Fig. 3). For example, for a planar framework of struts (denoted by the dotted lines in Fig. 3), these quantities are computed from

$$p_{ij}^v = \frac{1}{2} \sum_{c=1}^3 \left(r_i^{cv} R_j^{cv} + R_i^{cv} r_j^{cv} \right), \quad (4)$$

where i and j are indices that run over x and y and the vectors \vec{r}^{cv} connect at the midpoints of the struts that emanate from junction (vertex) v . These vectors circulate the junction in the anticlockwise direction and make loops around the voids, which they circulate in the clockwise direction (see Fig. 3). The vectors \vec{R}^{cv} point from the center of the triangle of vectors around v to the center of one of its neighbor loops c . This fabric tensor is symmetric, $p_{ij}^v = p_{ji}^v$, and discrete, namely, it takes a specific value at each junction. The coarse-graining of this tensor leads to a continuous description of the structure, $p_{ij}(\vec{r})$. In terms of this tensor the closure equation in two dimensions is

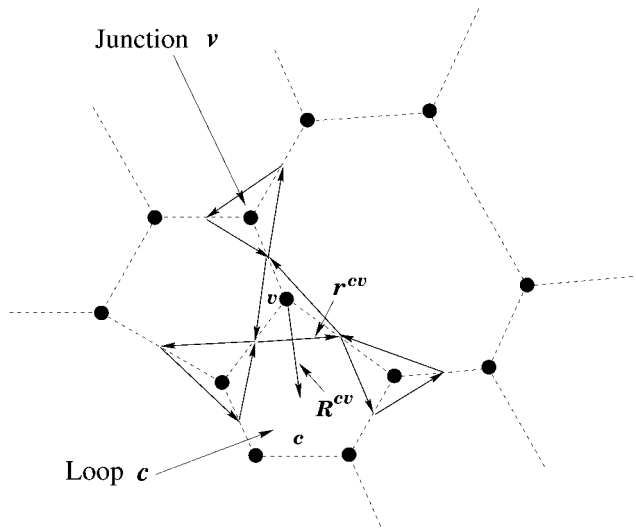


FIGURE 3 Example of the structural characterization in a two-dimensional framework of struts. The vectors \vec{r}^{cv} connect midpoints of the struts around junction v , circulating in the anticlockwise direction. The vector \vec{R}^{cv} extends from the center of triangle v to the center of one of its neighbor loops, c . The symmetric part of each term in the tensor $\hat{C}^v = \sum \vec{R}^{cv} \vec{r}^{cv}$ gives the components of the fabric tensor p_{ij} , which couple to the stress tensor in Eq. 5. This equation and the balance conditions 1–3 form a closed set of stress field equations.

$$p_{yy}\sigma_{xx} + p_{xx}\sigma_{yy} = 2p_{xy}\sigma_{xy}. \quad (5)$$

Equations 1–3 and 5 form the stress field equations for two-dimensional continuous isostatic media. Although the field equations seem difficult to analyze in their present form, it is possible to decouple them, using an expansion in the gradients of the fields p_{ij} , which turn out to decrease strongly with lengthscale. To lowest order, this gives the following explicit equations for the stress components (36)

$$\left(p_{xx} \frac{\partial^2}{\partial x^2} + 2p_{xy} \frac{\partial^2}{\partial x \partial y} + p_{yy} \frac{\partial^2}{\partial y^2} \right) \sigma_{ij} = f_{ij}, \quad (6)$$

where the terms f_{ij} are known functions of the components of the fabric tensor and of the gradients of the external forces \vec{g} . The exact derivation of Eq. 6 is not essential for this discussion; what is significant is that it is hyperbolic! This means that the solutions of Eq. 6 for localized stimuli do not spread uniformly but rather propagate along *narrow channels*. Such a set of hyperbolic field equations and their solutions are termed here “isostaticity theory”.

To illustrate the narrow-channel solutions in a particular system, consider an isostatic medium occupying the semi-infinite plane $x \geq 0$, as sketched in Fig. 4. Let us presume that on the boundary of this system, $x = 0$, there acts a localized stimulus consisting of a force in the x direction, and choose the point around which the force is localized as $A = (0, 0)$. The boundary data is most conveniently represented in terms of stresses, $\sigma_{xx}(x = 0, y) = U(y)$, and their gradients,

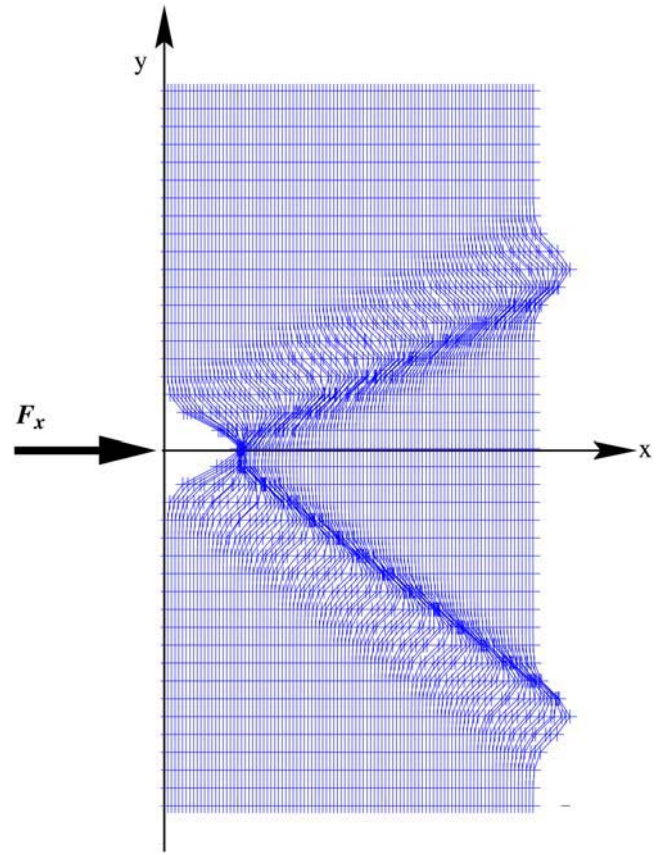


FIGURE 4 Example of the propagation of a bell-shaped stimulus at the boundary into a two-dimensional isostatic medium. The medium is continuous and the magnitude of σ_{xx} is plotted at discrete points in the material. The values of the components of the geometric tensor are taken to be random. Note that 1), the propagation need not be symmetric around $y = 0$; and 2), the amplitudes of the forces along the two channels need not be the same.

$\partial_x \sigma_{xx}(x = 0, y) = V(y)$, where $U(y)$ and $V(y)$ are arbitrary functions that, to mimic a localized stimulus, are narrowly distributed around A . The imposition of the boundary data on σ_{xx} and $\partial_x \sigma_{xx}$ is chosen for its consistency with the hyperbolic nature of Eq. 6. To make contact with experimentally measured forces, note that if the stimulus acts over a boundary section of size d then the force F_x in the figure is $\int_{-d/2}^{d/2} \sigma_{xx} dy$. For simplicity, I also assume that the external field \vec{g} is constant in space (e.g., gravity). The general solution of the field equations under this stimulus is

$$\sigma_{xx} = \frac{1}{2} \left[U \left(\frac{S^0}{p_{xx}^0} \eta \right) + U' \left(\frac{S^0}{p_{xx}^0} \zeta \right) \right] + \frac{1}{2} \int_{\eta}^{\zeta} \left[V \left(\frac{S^0}{p_{xx}^0} t \right) + \frac{p_{xy}^0}{p_{xx}^0} U' \left(\frac{S^0}{p_{xx}^0} t \right) \right] dt, \quad (7)$$

where $S = \sqrt{p_{xy}^2 - p_{xx}p_{yy}}$ and $U' = dU(s)/ds$ is the derivative of U with respect to its argument. S^0 and p_{ij}^0 stand for the values of these quantities along the boundary $x = 0$. The coordinates η and ζ are linear combinations of x and y

$$\eta = -\left(1 + \frac{p_{xy}}{S}\right)x + \frac{p_{xx}}{S}y\zeta = -\left(1 - \frac{p_{xy}}{S}\right)x + \frac{p_{xx}}{S}y, \quad (8)$$

where the coefficients of x and y depend only on the geometric characteristics p_{ij} . The solution (7) shows that the boundary stimulus propagates into the medium via two characteristic lines, $y_\eta(x)$ and $y_\zeta(x)$, along which the values of η and ζ are constant. For example, suppose a stimulus of magnitude σ^0 has a bell-like form and is localized within an area of size d on the boundary,

$$U(y) = \sigma^0 e^{-y^2/2d^2} \quad \text{and} \quad V(y) = 0. \quad (9)$$

The explicit solution for the stress field in the medium for this loading is

$$\sigma_{xx} = \frac{\sigma^0}{2} \left[\left(1 + \frac{p_{xy}}{S}\right) e^{-\frac{S^2 \zeta^2}{2d^2 p_{xx}^2}} + \left(1 - \frac{p_{xy}}{S}\right) e^{-\frac{S^2 \eta^2}{2d^2 p_{xx}^2}} \right]. \quad (10)$$

The solution consists of two bell-shaped peaks that propagate into the medium, as shown schematically in Fig. 4. The peaks of the signals are centered on the two lines

$$y_\eta = \frac{S + p_{xy}}{p_{xx}}x \quad \text{and} \quad y_\zeta = \frac{S - p_{xy}}{p_{xx}}x. \quad (11)$$

The forces that propagate along these lines can be calculated by first using Eq. 11 to find the local unit tangents to these lines, \vec{t}_η and \vec{t}_ζ , and then carrying out the following integrals

$$F_\eta = \int_{-d/2}^{d/2} \vec{\sigma} \cdot \vec{t}_\eta dy \quad \text{and} \quad F_\zeta = \int_{-d/2}^{d/2} \vec{\sigma} \cdot \vec{t}_\zeta dy.$$

This class of nonuniform solutions provides an effective mechanism for transmission of the force from the stimulus point into the cell via two narrow channels, along which the forces *hardly attenuate*. Although the trajectory of a channel may fluctuate locally, its general direction is well defined. For example, the trajectories drawn in Fig. 4 were computed for random values of the p_{ij} s, chosen from a broad distribution. The amplitude of the fluctuations was 0.1 with a standard deviation of 0.08. Yet, the lines appear almost perfectly straight. This feature makes it possible to identify the origin of the stimulus force anywhere along the transmission channel from the local gradient. Another significant feature of this model is that, given knowledge of the boundary stimulus at point A and of the (possibly temporal) structure of the network, it is possible to determine a priori the exact paths that the forces would take from A. This has possible ramifications on the dynamics of reorganization of the CSK (see discussion in the concluding section). It is also possible to terminate one of the paths by local reorganization of the CSK structure, as will also be discussed below, giving the cell even greater control over the force transmission.

A caveat to the analysis is that, at present, isostaticity theory has only been developed explicitly in two dimensions. The three-dimensional theory, where three “missing” conditions are required to close the stress equations, has not yet made an appearance in the literature. Nevertheless, these equations have been derived recently by this author, as will be reported shortly. Initial calculations of simple networks indicate that the three-dimensional equations support a similar type of nonuniform solution.

The above discussion provides a potential dream model for intracellular stress transmission: 1), it is conveniently continuous, yet it describes communication of forces via narrow channels; 2), the model gives rise to a nonuniform transmission of signals that describes the action at a distance observed experimentally; 3), forces hardly attenuate along the channels, giving very good signal/noise ratios; 4), the narrow channeling makes it possible for the cell to exercise good control over the signal; and 5), the predictability of force path directions both enables the modeller to predict the exact stresses that develop in the CSK and allows the cell itself to exploit this feature as an input for the reorganization dynamics of the CSK structure.

It follows that an isostatic, or near-isostatic, structure of the CSK would make possible a good control over mediation of forces and provide an explanation for several difficult experimental observations. But is the CSK close to an isostatic state? This author could find no direct experimental evidence that would provide a clear-cut answer to this question. Such evidence is essential if we wish to benefit from the advantages that this model offers. To provide a basis for testing this issue, it is important to quantify the structural characteristics that need to be observed if the CSK is in such a state. This is done in the next section.

The conditions for isostaticity of the CSK

Of the three primary types of filaments, AFs, IFs, and MTs, it is commonly believed that the former two support only tensile forces. However, it is worth keeping in mind that bending forces can be ignored only if they give rise to sufficiently weak torque moments. Whether this is truly so depends on the force magnitude and the filament rigidity. In particular, sufficiently short and thick bundles of AFs may be able to support weak bending forces. When, or if, that happens these must also be regarded as stiff elements. Note that this type of sensitivity to the magnitude of the bending force has no analog in conventional continuous theories, another fundamental difference between the two approaches.

In contrast, MTs, which are hollow and stiff cylindrical filaments, are able to support relatively large compressive and bending forces. Consequently, they are usually regarded as rigid struts. Note that struts need not necessarily be straight.

AFs typically form junctions of two kinds: 1), cross-links between two linear filaments, in which case four arms come out of the junction and it is called quadrivalent; and 2),

branching points, in which case three arms emanate from the junction and it is called trivalent. The CSK framework can be regarded as a collection of elements, each extending between two neighboring junctions. Any one filament may consist of a number of elements, e.g., the filament in Fig. 5 *a*, which includes elements α , β , and γ .

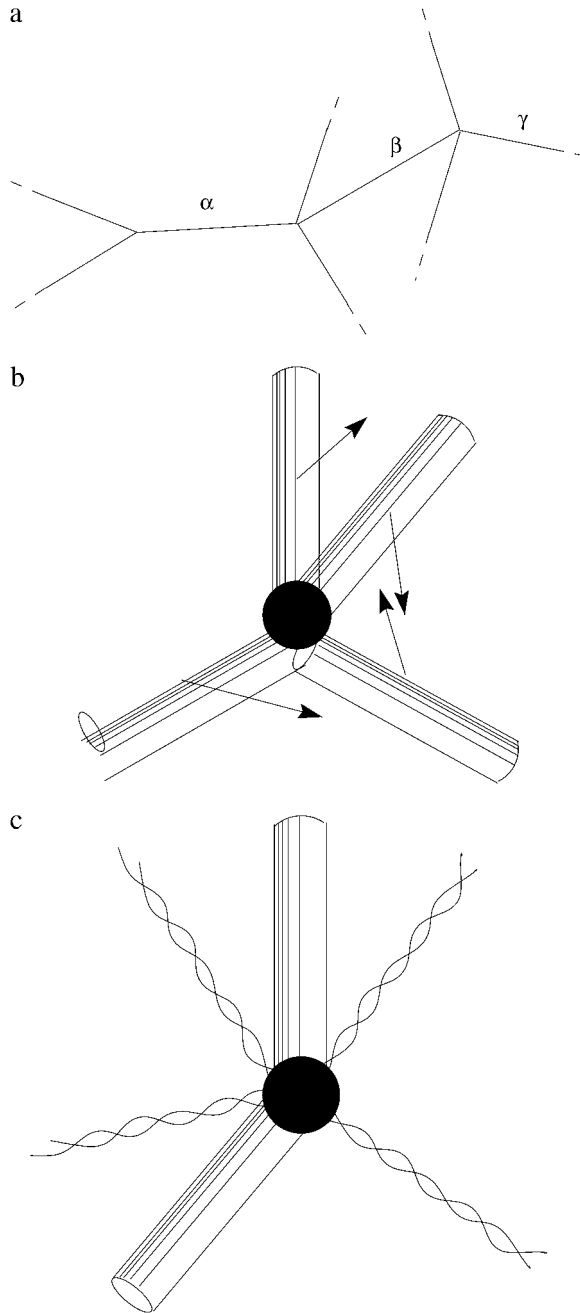


FIGURE 5 (a) One filament may consist of several elements. Elements are sections of the filament that are separated by junctions and transmit forces between them, e.g., α , β , and γ . (b) A junction connecting four rigid filaments can transmit bending forces and therefore torque moments. (c) A junction may join both cables and struts.

An element transmits a force between junctions. Cable elements transmit only longitudinal (tensile) forces, whereas struts transmit forces that have both longitudinal (tensile or compressive) and transversal (bending) components. The bending components give rise to torque moments around the junctions. A force vector between two junctions is therefore characteristic of the element between them and is termed in the following “element force”. An element can also transmit a torque moment. For the purpose of the following analysis, elements that transmit no force at all between junctions are not considered to be part of the framework.

For regions in the framework to be isostatic under external loading, even only temporarily, the forces that develop in the elements must match in number the equations that can be constructed around the junctions. In the following, I discuss the conditions for isostaticity in a large region containing N junctions. I consider three possible scenarios in increasing levels of generality.

Scenario A

Suppose that all the junctions between the filamentous components can support torque moments. This would be the case if most of the filaments could support bending forces. In this case many junctions would connect to relatively stiff struts, as sketched in Fig. 5 *b*. The plausibility of this scenario is low and it is presented here mainly for completeness and to illustrate the rationale of the argument for a relatively straightforward case. Nevertheless, it may also have practical relevance if some of the forces that develop in the CSK under working conditions are sufficiently weak. Although such a possibility is unlikely to occur in bead-pulling experiments, where forces are of the order of hundreds of pN, such a situation cannot be ruled out a priori in cells with sufficiently thick AF bundles.

Consider, then, a region of N junctions in mechanical equilibrium under an arbitrary set of external forces on its boundary. The loading gives rise to element forces, and for the structure to be in an isostatic state the forces should be determinable from balance equations. It is convenient to define the following structure. Around every junction, draw straight lines connecting the midpoints of the elements that connect to it, as sketched in Fig. 6. These lines make edges of polyhedra that surround the junctions and every polyhedron is taken to be connected at its corners by a joint with its neighbors. Under an external loading, this framework transmits forces between neighboring polyhedra v and v' , which are the analogs of the element forces $f_{vv'}$ between junctions v and v' in the original network (see Fig. 6). Each $f_{vv'}$ is a vector, which means that at every contact between neighboring polyhedra there are three unknowns to determine. The number of unknowns is then three times the number of struts, $N_{\text{unknown}} = 3S$. Denoting the valency of junction v by z_v and considering $N \gg 1$ (R. Blumenfeld, unpublished data), the total number of strut elements between junctions is

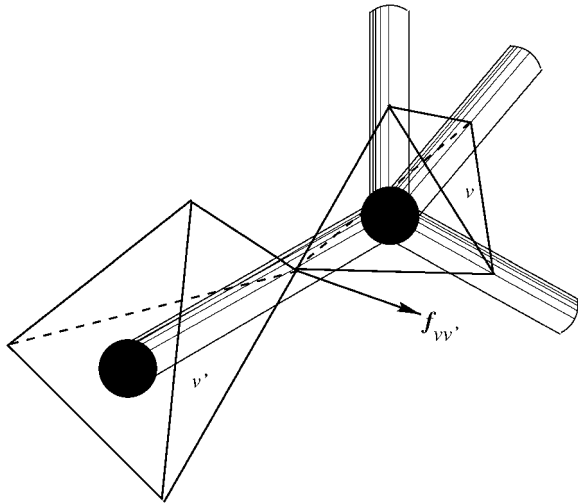


FIGURE 6 Construction of the framework of polyhedra. The edges of a polyhedron consist of lines extended between midpoints of elements meeting at the junction. Every polyhedron is considered to connect to neighboring polyhedra at its corners. There are three conditions of force balance around every polyhedron and, depending on the nature of the elements entering the junction, three conditions of torque balance.

$$N_{\text{elements}} = \frac{1}{2} \sum_{v=1}^N z_v \equiv \frac{1}{2} N \bar{z}, \quad (12)$$

where \bar{z} is the mean valency per junction. It follows that there are altogether $3N\bar{z}/2$ unknowns.

Now, each polyhedron is in mechanical equilibrium under the forces applied on it by its neighbors. Then, it must satisfy both balance of forces (three equations for the three space directions) and balance of torque moments (three equations for the three axes of rotations in three dimensions). This gives six equations per polyhedron and altogether $6N$ conditions. To be statically determinate we must have $3N\bar{z}/2 = 6N$, which gives that the only requirement that this structure should satisfy for it to be isostatic is $\bar{z} = 4$. It should be noted that the requirement is on the *mean valency* rather than on the valency of every junction, and therefore it is quite unrestrictive.

Note that if one presumes that the corners of the polyhedra were connected rigidly to one another, a situation that corresponds to subtly different boundary loading, the value of the mean valency comes out to be $\bar{z} = 2$, which gives an unphysical condition. A discussion of which situation is more appropriate is not only outside the scope of this analysis but also somewhat superfluous because of the implausibility of this scenario in the first place.

Scenario B

In the second scenario, I relax the above assumptions. Let now the range of forces be such that some of the AFs and IFs cannot support bending. In this case, these elements can be regarded as cables that support only tensile forces. An

element between any two neighbor junctions can be either a cable or a strut. Presume, for the moment, that the fraction of struts is sufficiently high so that every junction connects at least two struts. This means that all the junctions can support torque moments (see, e.g., Fig. 5 c). The likelihood of this scenario is again subject to the occurrence of sufficiently weak intracellular forces in some elements under working conditions, as discussed above.

The difference between this and scenario A is that cable-like elements can have only one unknown, the magnitude of the tensile force that aligns in the direction of the cable. Struts support forces that can point in arbitrary directions plus torque moments, giving six unknowns per strut. Denoting the total number of strut and cable elements in the structure— S and C , respectively—the total number of unknowns is then

$$N_{\text{unknowns}} = 6S + C. \quad (13)$$

The total number of elements between junctions is again $\bar{z}N/2$,

$$N_{\text{elements}} = C + S = \frac{\bar{z}N}{2}. \quad (14)$$

Since every junction can support a torque moment, then, to be in mechanical equilibrium, each polyhedron must satisfy six equations as before, giving a total of $6N$ equations. It follows that for the structure to be isostatic the following equality must be satisfied

$$6N = 6S + C. \quad (15)$$

Solving from relations 14 and 15 for S and C , we obtain the following conditions for isostaticity:

$$S = \frac{12 - \bar{z}}{10} N \quad \text{and} \quad C = \frac{3(\bar{z} - 2)}{5} N. \quad (16)$$

Recall that this scenario requires that the number of struts around every vertex is at least two. A detailed analysis of this constraint, which will be done elsewhere, shows that this second scenario is not possible and that networks of struts and cables cannot be isostatic if all the junctions are rigid. Isostatic states can only be supported when some of the junctions are sufficiently soft and cannot support torques. This scenario is analyzed next.

Scenario C

In the third scenario the assumptions are relaxed further, making it the most general and the most realistic. The networks are now allowed to contain some junctions that cannot support any torque moment. Let these junctions comprise a fraction x of the total number of junctions N . The key difference between this and the previous scenario is that in mechanical equilibrium these xN junctions can only provide three equations of force balance each. The remaining $N(1 - x)$ junctions can still provide six balance equations each as before—three of force and three of torque. Therefore, the

total number of equations available to determine the forces is now $6N(1-x) + 3Nx = 3N(2-x)$. As before, there are six unknowns to determine at each strut and one unknown to determine at each cable, and relation 14 still holds. However, care must be taken when counting the C . Some of the xN junctions, which do not support torques, may still be connected to the end of exactly one strut. Being the only strut at a junction means that it can only support a longitudinal force, tension or compression. This means that despite being a stiff element, such a strut only gives rise to one unknown force component. Therefore, for the purpose of the analysis of this scenario, such a strut should be counted within C . Requiring, now, that the number of equations be equal to the number of unknowns gives

$$3N(2-x) = 6S + C. \quad (17)$$

Solving relations 14 and 17 gives that for static determinacy there should be the following numbers of struts and cables

$$S = \frac{6(2-x) - \bar{z}}{10}N \quad \text{and} \quad C = \frac{3(\bar{z} + x - 2)}{5}N. \quad (18)$$

To check this result for consistency, note that $x = 0$ reproduces Eq. 16. When $x = 1$, there are no torque-carrying junctions at all, which means that $S = 0$. Therefore, all elements carry forces that align along the direction of the filament, and Eq. 18 gives $\bar{z} = 6$. This is the tensegrity limit because the structure is made mostly of cables with occasional struts that are under compression between junctions.

Denoting the relative fraction of cables and struts in the structure α and $1 - \alpha$, respectively, makes it possible to deduce from the relations in Eq. 18 that the mean valency is

$$\bar{z} = \frac{2-x}{1-5\alpha/6}. \quad (19)$$

Relation 19 describes a surface in the three-dimensional space spanned by x , α , and \bar{z} , as shown in Fig. 7. The values

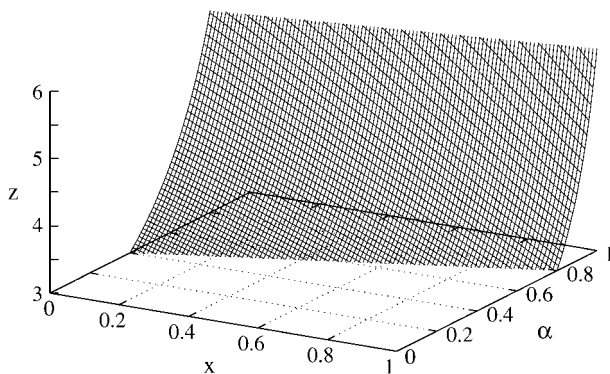


FIGURE 7 For the CSK structure to be isostatic, the mean valency \bar{z} is a given function of the fraction of torque-carrying junctions, x , and the fraction of cable elements, α , in the framework. This function describes a surface in the three-dimensional space spanned by x , α , and \bar{z} .

of α and x must be consistent with one another. It is evident that as $x \rightarrow 1$, $\alpha \rightarrow 1$, and as x decreases so does α , but the reverse statement is not true— α may be changed without affecting x . Therefore, there is no one-to-one relation between the two. It follows that not all regions on the surface shown in Fig. 7 are physically realizable. Nevertheless, the relation shows that the mean valency must lie within a limited range of values and this makes it possible to test this prediction experimentally.

DISCUSSION AND CONCLUSION

To conclude, this article has examined the paradigm that underlies modeling of intracellular stress transmission. It has been shown that conventional continuous models do not fail because of being continuous, but rather because of the Saint-Venant compatibility condition built into the equations that govern the stress field. This condition necessitates the introduction of deformation-based information and is directly responsible for the attenuation of stress fields away from a localized stimulus. It is this basic feature that makes these models inadequate for describing action at a distance.

It has also been argued that turning to discrete models as a way to overcome this difficulty is based on two misguided ideas: that discrete models would automatically give rise to nonuniform fields and that all continuous models would automatically give rise to diffuse stresses. Both these misconceptions have been put straight: 1), discrete models will give rise to stress fields that dissipate on scales larger than the mesh size unless their structures satisfy specific conditions; and 2), continuous models may give rise to nonuniform force channeling as long as the field equations are hyperbolic. Thus, while discreteness may be a necessary condition for the focusing and directing of forces on the element scale, it is by no means sufficient to maintain directionality over large distances.

A new model has then been proposed, based on the idea that action at a distance and channeling of force stimuli is possible in a continuous model if considerable parts of the CSK structure were isostatic. This concept has been explained and isostaticity theory has been briefly reviewed in two dimensions. This theory provides a promising model for the transmission of intracellular forces due to several advantages: 1), it offers the convenience of continuous modeling and hence the ability to describe stresses on lengthscales larger than the size of the mesh of the CSK; 2), it gives rise to nonuniform stresses and to propagation of forces into the cell via narrow channels, which explains the good control that cells have over transmission of mechanical signals; 3), it explains the experimental observations of action at a distance; and 4), given a specific force stimulus, the model makes it possible to predict the path along which the force focuses as it propagates into the cell.

Next, I discussed the requirements of the CSK to be fully isostatic. The most important condition is that the mean

valency \bar{z} must have a specific value. This value depends on two quantities: 1), α : the fraction of cable elements in the structure; and 2), x : the fraction of “soft” junctions that cannot support torque moments. The structural conditions for isostaticity have been analyzed quantitatively for several possible scenarios and it has been concluded that the most realistic case must involve finite values of both x and α . A general expression relating x , α , and \bar{z} has been derived.

Before discussing the implications of the analysis, it is useful to clarify the relations between the model presented here and the concept of prestress (39). Most cells are under the influence of external loading, sometimes termed contractile forces or traction field (24). These forces give rise to a stress state comprised of a distribution of tensile forces in the filaments of the CSK network. This is the prestress. In the language of mechanics, the contractile forces, which normally act on extensive parts of the boundary, constitute an initial boundary loading. A stimulus is normally an additional force applied to the boundary which is usually quite localized, e.g., on a focal adhesion point. The stimulus has two effects: it modifies the boundary loading, simply because the entire structure has to maintain mechanical equilibrium on the timescales involved, and it changes the internal stresses. When treating such situations in mechanics, the usual approach is to start from a simplifying assumption on the modification of the boundary loading and to solve for the new stress state. This is general to any mechanical model. The only difference between my model and any other is in the form of the solution. Since many of the filaments are not rigid, then prestress is essential to the formation of a stress-bearing structure in the first place. The analysis presented above already presumes the existence of such a structure and hence of prestress. Moreover, within the model presented here, given the boundary contractile forces and the structure of the network it should be possible to calculate the prestress. Then, given the additional stimulus and the modified boundary loading (for example, it can be assumed that this change is small and distributed uniformly across the boundary), it is possible to calculate the transmission of the stimulus on top of the prestress.

This picture agrees very well with several experimental observations. It has been observed that action at a distance is sensitive to prestress (18,19,24). This can be understood by considering what generally happens to a channeled force as the prestress is changed slightly. The change can have one of three effects on the isostatic structure: 1), no filaments tighten or slacken and only internal stresses and strains change; 2), loose filaments tighten; and 3), taut filaments slacken. In case 1, the topology of the system remains intact but junctions of the structure displace slightly, giving rise to small changes in the values of the p_{ij} . Since the topology is unchanged, the structure remains isostatic and the change does not affect the force channeling beyond small modifications of magnitude and trajectory. Therefore, this case still supports action-at-a-distance. In case 2, the tightening of

filaments adds elements to the structure and changes the topology. In particular, this increases the value of \bar{z} and increases the number of unknowns. This upsets the balance between unknowns and equations, rendering the unknowns under-determined. This leads to dispersion of the stimuli through the structure, which can be understood both on the mesh and on the continuous levels. If more elements come out of a junction, then a force coming into the junction exits it more dispersed. On the continuous level, the under-determinacy requires extra conditions and these are provided by the compatibility conditions. This gives rise to locally elliptic continuous stress equations whose solutions are attenuative as in conventional elasticity theory. Thus, the change in topology undermines the isostatic solutions and the force chains disintegrate. This explains the observations of the sensitivity of action at a distance to elevation of the prestress. In case 3, the topology changes as well, because, for the purpose of stress transmission, slackened elements cease to be part of the structure. This changes again the numbers of elements and junctions, upsetting isostaticity in regions of the network. Consequently, the force channels are no longer the correct solutions in the vicinity of slackened elements, and they disintegrate. This explains why prestress is necessary for the stability of the CSK (24). Hu et al. reported experiments where the tensile stresses in the CSK were modified by over-expression of caldesmon (18). The caldesmon decorates AFs and inhibits their tension, which leads to local slackening of elements. At some level of caldesmon they have detected regions of concentrated strain. In this experiment the strain should be understood only as a means to measure stress, and therefore such concentration indicates force channeling. On reducing the over-expression of caldesmon, they observed emergence of more regions of high strain. This is the same sensitivity to prestress discussed above: on increasing internal prestress, more elements have joined the force-carrying network, giving rise to stresses away from the initial focused channels. It is interesting to note that, in addition to the appearance of new stressed regions, their images show that initially focused stresses also disappear (compare the strains at coordinates $(x, y) = (-15, -5) \mu\text{m}$ between Figs. 3 C and 3 E of Hu et al. (18)). This inverse response to increasing local tensile forces cannot be explained by any conventional theory, but it is indicative of isostatic behavior. All these observations lend strong support to the isostatic model.

The analysis presented in this article highlights an issue that has no analog in conventional models. Whether a structure is isostatic depends crucially on the distribution of its cable and strut elements. But IFs and AFs, which behave as cables under typical forces of bead-pulling experiments, may behave as struts under much weaker forces, especially if bundles of AFs are present. This raises an important question: could intracellular forces under working conditions be sufficiently weak in cells to allow some AF bundles to behave as torque-bearing struts? If this is so, then

bead-pulling and twisting experiments may be missing the point by measuring at force magnitudes that wash out very relevant phenomena, and such measurements should be reexamined. Note that in conventional models the magnitude of the forces cannot play such a conceptual role.

Another point that should be emphasized is that for the cell to exert the type of control on stress transmission described here, the CSK structure need not be fully isostatic. It is not inconceivable that the structure of the CSK, which is constantly changing and reorganizing, may satisfy these conditions only approximately and so be just nearly isostatic. The proximity to perfect isostaticity is measured by the discrepancy between the number of unknown forces and the number of balance equations that can be constructed for them. If the discrepancy is not too large compared to the number of junctions, then the CSK consists of considerable regions that are locally isostatic. In these regions, force transmission would be channeled along narrow paths. The smaller the discrepancy, the larger the isostatic regions, the closer the structure is to being isostatic, and the longer the force channels. This would lead to minimal attenuation and cells would have better control over the mediation of forces. Ultimately, the proximity of the CSK to an isostatic state is determined by the combination of the mean valency and the ratio of cables to struts. If the mean valency is too low relative to \bar{z} , then a stable structure cannot be sustained at all. If, on the other hand, too many junctions connecting microtubular elements have high valencies then the framework is over-connected and too far from an isostatic state. This would lead to deterioration of the control over force transmission, effecting a behavior characteristic of conventional materials. Forces applied at the focal adhesion points would then diffuse and attenuate as they penetrate the cell. Regular periodic structures are usually in this regime due to their high symmetry. Consequently, the relevance of models that assume a lattice-like structure should be regarded with caution. The ability to control proximity to isostatic states could be a considerable advantage from a designer's point of view. For example, the dependence on a global mean of the valency rather than local details means that the ability to channel and control forces is not over-sensitive to local structural fluctuations.

This leads to an interesting speculation: an effective strategy for the cell would be to maintain a CSK structure that is not exactly isostatic but only predominantly so. This allows us to have judiciously selected small regions of locally high mean valencies that behave either elastically or viscoelastically. By controlling the size and nature of such regions, it is possible both to maintain good control over the paths along which forces propagate and to admit preferential deformations that can accommodate other cell functionalities. Entertaining small "elastic" regions has another advantage. Recall that in response to a localized force stimulus, an isostatic medium generates a pair of force channels. But the cell only needs one such signal to reach the vicinity of the

nucleus. Placing a high- z region in the path of one of the channels dissipates the focused force and effectively terminates it. Thus, the manipulation of local connectivity provides several important mechanisms to control force propagation into the cell.

Many of these ideas lead to direct predictions, and therefore they can be tested experimentally. One test would be a determination of the proximity of CSK structures to isostatic states. To obtain quantitative data on this issue, measurements are needed of the mean valency \bar{z} and of the relative fractions of cables to struts α . For the latter, one needs to use the known mechanical properties of AFs and AF bundles to estimate whether in a given structure they should be regarded as cables or not under given working conditions. Using those measurements and relation 19 in inverted form,

$$x = 2 - (1 - 5\alpha/6)\bar{z}, \quad (20)$$

it is possible to evaluate x . This value can then be checked against independent measurements of the torque moments that junctions of the network can support. There is a possibility that such measurements can be simplified if we consider that only some regions in the CSK need to be isostatic. The simplification is that the measurements need not involve the entire CSK but only selected relevant sections where action at a distance is observed.

Another set of experiments should aim to estimate the fraction of junctions $(1 - x)$ where more than two MTs meet. Although some of this information may exist in the literature, the data needs to be reassessed, taking into consideration the present definition of elements.

Another exciting set of experiments should focus on the dynamics of reorganization of the CSK. If the picture proposed here is correct and isostatic states indeed play a significant role in intracellular stress transmission, then it must have implications regarding the dynamics of the CSK. In particular, one possible aim of the reorganization process may be to direct force transmission through control of local isostaticity. A potential mechanism may be the following. As a force is applied to the boundary of the network it initially propagates into the cell roughly in the direction of the interior. By judicious polymerization of filaments, following mechanochemical cues, the structure can be remodeled into generating locally isostatic regions that channel the force toward an intended destination. Another goal of structural remodeling would be to undermine local isostaticity, as discussed above. Thus, experiments to investigate this issue should aim to determine correlations between reorganization, connectivity changes, and force channeling. This type of experiments would be a significant step on the road to build a general model that includes the slower biochemical reactions.

From a designer's point of view, structural reorganization better be localized for several reasons: one is for economy in energy and raw materials; another is that the larger the reorganization the larger the chemical activity involved, and therefore the longer the response time; and finally, large

reorganization processes may affect adversely other ongoing functions of the CSK, such as being a highway for enzyme motors. Thus, maintaining “dynamic local isostaticity” is probably more efficient than managing a global isostatic structure.

Another point to note is that a constant change of the entire CSK structure is not economical. To use this mechanism effectively, large parts of the CSK network must remain relatively fixed, or change on relatively long timescales. It is around these stable parts that the CSK structure needs to be dynamically manipulated. It is therefore tempting to conjecture that this may be one of the roles of the intermediate filaments: to act as relatively stable elements around which rearrangement is much more dynamic. This issue, as well as all the speculations on the dynamics of rearrangement, is amenable to experimental testing.

Another interesting set of possible experiments is *in vitro*. I propose that construction of artificial CSK-like mixtures of AFs, IFs, and MTs in the laboratory may be a useful way to test these ideas in a controlled fashion. Initially the experiments should be done on two-dimensional specimens, at least until sufficient insight is gained on three-dimensional systems. The prediction is that force channeling should be observed when the right structural conditions for isostaticity are met. It should be commented that although conceptually these conditions can be derived in exactly the same way as above, the quantitative values of the mean valency would be different due to the different dimensionality. For example, under the conditions of the third and most general scenario, the numbers of struts and cables is, respectively,

$$S = \frac{6 - 2x - \bar{z}}{4}N \quad \text{and} \quad C = \frac{3\bar{z} + 2x - 6}{4}N. \quad (21)$$

Two-dimensional model experiments *in vitro* have the advantage of easy visualization and therefore it would be also interesting to analyze the dynamic response of such a network to stimuli in general and to the channeled forces in particular.

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