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Long-distance propagation of forces in a cell

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Abstract

A fundamental question in the field of mechanotransduction is how forces propagate inside a cell. Recent experiments have shown that a force of a physiological magnitude, applied via a focal adhesion, can propagate a long distance into the cell. This observation disagrees with existing models that regard the cell as a homogeneous body. We show that this "action at a distance" results from the inhomogeneity in the cell: a prestressed and stiff actin bundle *guides* the propagation of forces over long distances. Our models highlight the enormous ratios of the prestress and the modulus of the actin bundle to the modulus of the cytoskeleton network. For a normal cell, the models predict that forces propagate over characteristic lengths comparable to the size of the cell. The characteristic lengths can be altered, however, by treatments of the cell. We provide experimental evidence and discuss biological implications.

Keywords: Cytoskeleton; Microfilaments; Microtubules; Intermediate filaments; Mechanotransduction

Evidence has accumulated that mechanical forces can regulate essential cellular activities such as gene expression, protein synthesis, and cell proliferation [1,2]. Exactly how the mechanical forces link to the cellular activities, however, remains elusive. Recent experiments have shown that a force of a physiological magnitude, applied via integrins at a small spot on the surface of a cell, can propagate a long distance into the cytoplasm and to the nucleus [3–6]. This "action at a distance" disagrees with models that regard the cell as a homogeneous body. For example, the continuum elastic model [7], as well as the elastic membrane models [8–10], predicts that a force applied locally decays over a short distance in space.

We suggest that the long-distance propagation of forces originates from the inhomogeneity of a cell. An actin bundle is prestressed and stiff, embedded in a compliant CSK network (Fig. 1). If the prestress of the bundle is much larger than the elastic modulus of the network, when subjected to a transverse force, the bundle moves like a violin string. Similarly, if the modulus of the bundle is much larger than that of the CSK network, a force along the bundle can propagate a long distance. That is, the actin bundle acts as a *force guide*.

On the basis of this picture, we propose two models: the *prestressed string model* for transverse motion and the *stiff fiber model* for longitudinal motion. For a normal cell, the models predict that the forces propagate over characteristic lengths comparable to the size of the cell. The characteristic lengths can be altered, however, by changing the prestress or the modulus of the actin bundle. We carry out experiments by twisting a magnetic bead attached on the surface of a living cell and imaging the displacement field in the cell. We show that our experimental observations are consistent with these models.

Methods

Human airway smooth muscle cells were used in the experiments. The cells were transiently transfected with yellow fluorescent protein

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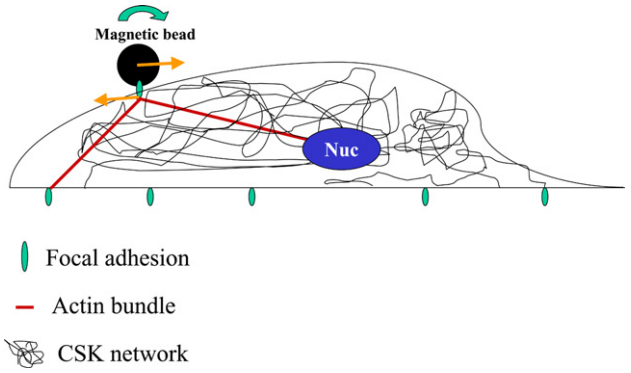

Fig. 1. On the apical surface of the cell adherent to a substrate, a magnetic bead connects to a preexisting actin bundle via integrins. When the bead is twisted, the actin bundle guides forces to propagate into the cytoplasm and to the nucleus. The compliant CSK network gently resists the motion of the actin bundle. A typical cell is ~5 μm high and ~100 μm long (not drawn to scale).

(YFP)-actin, YFP-cytochrome *c* oxidase (in mitochondria), and green fluorescent protein (GFP)-caldesmon. The cells were plated on two types of substrates: an extracellular matrix (ECM) and a poly-L-lysine coated dish. A magnetic bead, diameter ~4.5 μm, coated with saturated amounts of Arg-Gly-Asp-containing peptides, was placed on the apical surface of a cell. The bead was subjected to an oscillatory torque using a magnetic twisting cytometer (50 G at 0.3125 Hz). During a twisting cycle, multiple fluorescent images of the cell were taken. Using image correlation, we obtained displacements of resolution ~5 nm. Further experimental details can be found elsewhere [5,6,11].

Results

Models

Prestressed string model

To analyze the transverse motion, we model an actin bundle as a string anchored at two ends, and the CSK network as a compliant matrix (Fig. 2A). The string is under a prestress σ_b and lies along the x direction. Subject to a transverse force, the string deflects into a profile $v(x)$. Consider the balance of forces in the transverse direction of an element dx of the string. The prestress exerts a force $h_b^2\sigma_b\partial^2 v/\partial x^2\,dx$ on the string, where h_b is the thickness of the actin bundle. The CSK network resists the deflection of the string, exerting a restoring force estimated by $(G_m v/h_m)h_b\,dx$, where G_m is an elastic modulus of the network, and h_m is the thickness of the network between the actin bundle and the substrate. The balance of the forces requires that

$$\partial^2 v/\partial x^2 = v/L_1^2, \tag{1}$$

with the characteristic length

$$L_1 = \sqrt{\sigma_b h_b h_m/G_m}. \tag{2}$$

The general solution to Eq. (1) is $v(x) = A\exp(-x/L_1) + B\exp(-x/L_1)$. The constants A and B are determined by the boundary conditions. The qualitative behavior depends on the characteristic length L_1 relative to the length of the actin bundle, l. When $L_1 \gg l$, the restoring effect of the CSK network is negligible, so that the actin bundle behaves just like a violin string: plucked in the middle, the whole string moves. When $L_1 \ll l$, the restoring effect of the CSK is significant, so that the deflection decays exponentially from the point of action, with the characteristic length L_1. Consequently, the distance over which the force propagates is limited either by the length of the actin bundle, or the characteristic length L_1, whichever is smaller.

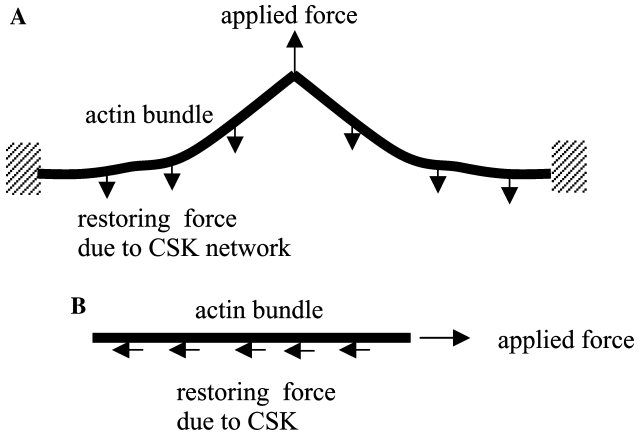

Fig. 2. Schematics of the prestressed string model for transverse motion (A), and the stiff fiber model for longitudinal motion (B).

Stiff fiber model

We analyze the longitudinal motion of an actin bundle by using a shear lag model, which has long been used in materials science and geophysics (see [12] for the literature). Let $u(x)$ be the displacement and $\sigma(x)$ be the tensile stress along the axis of the actin bundle (Fig. 2B). We model the actin bundle as an elastic fiber, obeying Hooke's law $\sigma = E_b\partial u/\partial x$, where E_b is the modulus of the actin bundle. As the actin bundle is pulled in the axial direction, the CSK network resists the motion of the actin bundle. We model this resistance by a distribution of shear stress estimated as $\tau = G_m u/h_m$. The balance of the forces along the direction of the actin bundle requires that

$$\partial^2 u/\partial x^2 = u/L_2^2 \tag{3}$$

with the characteristic length

$$L_2 = \sqrt{E_b h_b h_m/G_m}. \tag{4}$$

The solution to Eq. (3) takes the same form as that of the transverse deflection. When $L_2 \gg l$, the restoring effect of the CSK network is negligible, and the force does not decay along the actin bundle. When $L_2 \ll l$, the restoring effect of the CSK network is significant, and the longitudinal displacement decays exponentially from the point of action, with the characteristic length L_2.

Model predictions

A typical actin bundle has modulus $E_b = 2.5 \times 10^6$–133×10^6 Pa and thickness $h_b \sim 200$ nm [13]. The modulus of the CSK network is $G_m = 10^2$–10^3 Pa [14–16]. After being isolated from the living cell, an actin bundle shortens to about a quarter of its initial length [17]. We estimate that the prestress in a single actin bundle is $\sigma_b = 2 \times 10^6$–100×10^6 Pa. These values give enormous ratios: σ_b/G_m and $E_b/G_m = 10^3$–10^5. Using representative values, $h_b = 0.2$–0.3 μm and $h_m = 3$–4 μm, we find from Eqs. (2) and (4) that the two characteristic lengths are ~100 μm. This length is comparable to the length of the cell. Consequently, for a normal cell, both models predict that the CSK network has a negligible effect on the motion of the actin bundle. When an actin bundle is plucked by a force F, the transverse displacement is

$$v(x) = Fx/(2h_b^2\sigma_b) \quad (5)$$

at distance x from the anchor. When an actin bundle is pulled by a force F, the longitudinal displacement is

$$u(x) = Fx/(h_b^2 E_b). \quad (6)$$

Experimental results and comparison with the models

For a normal cell, our models predict that a locally applied force can propagate over a long distance along an actin bundle. This is exactly what we observed in a living smooth muscle cell (Fig. 3 and Supplementary Movie). When the magnetic bead was twisted, the actin bundles attached via integrins to the bead moved like strings within the entire field of view (>30 μm). This observation is consistent with the prestressed string model, but disagrees with the conventional homogeneous cell models.

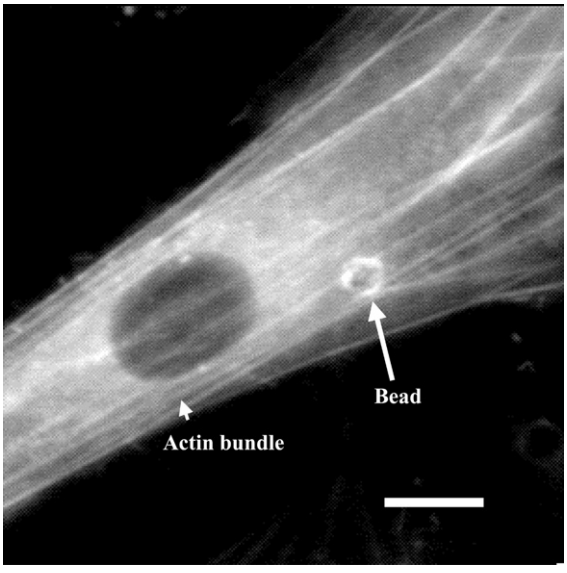

Fig. 3. A fluorescent image of a YFP-actin transfected smooth muscle cell (adapted from Ref. [5] with permission). A magnetic bead applies the load to the cell via focal adhesions, displacing the actin bundles. Displacements were observed at distances 20–30 μm from the bead (see Supplementary movie). The scale bar = 10 μm.

As another prediction of our models, if the bead is at the edge of a cell, where an actin bundle is anchored to the substrate, the displacements will be very small (Eqs. (5) and (6)). If, however, the bundle is anchored at one end to the nucleus and loaded at the other end, the displacements will be transmitted from the point of action into the nucleus with little decay. Both predictions were confirmed in experimental observations [18].

As mentioned above, in a normal cell, the restoring effect of the CSK network is negligible, so that the displacements of an actin fiber follow Eqs. (5) and (6). Subject to forces of a comparable magnitude in the transverse and the longitudinal directions, the corresponding displacements scale as $v/u \sim E_b/\sigma_b$. Experimentally, we observed that the transverse displacements were typically several times the longitudinal displacements. This anisotropy was also observed in a previous experiment [6]. These observations suggest that the modulus of the actin bundles should be several times the prestress. Available experimental data are not precise enough to ascertain this prediction.

The models also predict that, in the absence of prestressed actin bundles, movements in the cell will be localized around the magnetic bead. To test this prediction, we compared a cell plated on an ECM and a cell plated on a poly-L-lysine-coated dish. The cell on the ECM expressed long actin bundles, guiding forces to propagate over long distances (Fig. 3). The cell plated on poly-L-lysine, however, did not form long actin bundles (Fig. 4, left image), leading to a localized displacement field (Fig. 4, right image).

Eq. (2) predicts that if σ_b is preferentially decreased relative to G_m, the characteristic length L_1 will decrease. To test this prediction, we overexpressed a low level of caldesmon, a known inhibitor of actomyosin interaction [19]. This inhibitor caused the displacement field to localize around the magnetic bead (Fig. 5, left image). To counteract the effect of caldesmon, calcium ionophore A-23187 (5 μg/ml for 10 min) was added to the cell. After the drug treatment, in the same cell, in response to the same load, large displacements occurred remote from the magnetic bead (Fig. 5, right image). The cell before and after the drug treatment exhibited similar numbers and patterns of actin bundles [5], suggesting that it was the prestress in the actin bundles, rather than the number of actin bundles, that was decreased by the overexpression of caldesmon and then restored by A-23187 treatment.

Discussions

Our models assume that the prestressed, stiff actin bundles guide the propagation of forces, while the

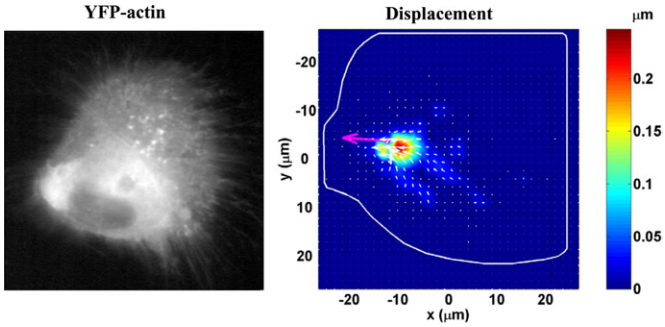

Fig. 4. Left: a fluorescent image of a living cell transiently transfected with YFP-actin plated on poly-L-lysine coated dishes; no long actin bundles could be observed. Right: a displacement map of a cell transfected with YFP-mitochondria and plated on a poly-L-lysine coated dish. The pink arrow represents the displacement direction of the center of the bead. The white arrows and the colors represent directions and magnitudes of the displacements of the CSK. The white lines are the boundaries of the cell.

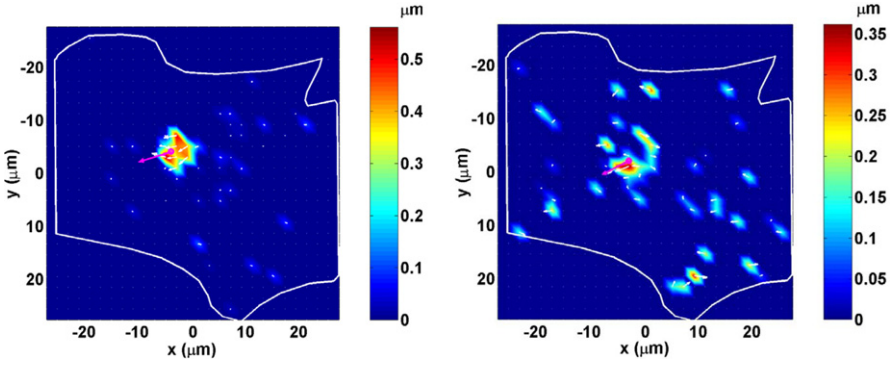

Fig. 5. Images of the displacement field of a cell transfected with a low level of GFP-caldesmon, before (left image) and after (right image) the treatment with calcium ionophore A23187 (5 μg/ml for 10 min), an inhibitor of caldesmon. The prestress was low with caldesmon overexpression, leading to localized displacements (left image). When the prestress was restored, large displacements appeared at sites remote from the bead (right image). Notations are the same as in Fig. 4.

CSK network provides a compliant elastic restoring force against the motion of the actin bundles. How valid is this assumption, given that a living cell has a complex, dynamic CSK [20]? The cytosol has a viscosity of ~0.01 poise, too small to be a major resistance to the motion of the actin bundles at physiological loading frequencies of 0.01–10 Hz. It is also known that the resistance to external loading comes mainly from the CSK rather than the membrane [11,21]. While the modulus of an individual actin filament or microtubule is about 1×10^9 Pa [22], the moduli of the networks of actin filaments, microtubules, and intermediate filaments are only of the order of 100–1000 Pa [23]. A similar order of magnitude has been measured for the effective modulus of living cells using various approaches (magnetic twisting cytometers, optical tweezers, and atomic force microscopes (AFM)) [14–16]. (Incidentally, modulus measured in living cells with an earlier form of AFM is of the order of 10^6 Pa [24], possibly due to the small tip of the AFM that probes the modulus of a single actin bundle.) Consequently, it is reasonable to assume that the modulus of the CSK network is much smaller than that of an individual actin bundle.

It is difficult to quantify exactly how much the overexpressed caldesmon alters the prestress in the actin bundles. A recent report has demonstrated that overexpressing a low level of caldesmon decreases the tractional forces by 50% without altering the number and the pattern of focal adhesions and actin bundles [19]. Since these actin bundles terminate at focal adhesions, through which the traction is exerted on the substrate [25], it is reasonable to assume that the prestress in the actin bundles on average is also decreased by 50%. Assuming that the treatment of A23187 negates the inhibition caused by caldesmon and completely restores the prestress in the actin bundles, the prestressed string model predicts that the drug treatment increases the characteristic length by ~30%. Fig. 5 shows large displacements at remote sites after the drug treatment, consistent with the prediction of the model.

The essence of our models is the incorporation of a prestressed, stiff actin bundle in a compliant CSK network. It is the enormous ratios of the prestress and modulus of the actin bundle to the modulus of the CSK network that allow a locally applied force to propagate over a long distance. By contrast, for a homogeneous

body, a force, applied at a small spot on the surface, is balanced by the stress field in the body; the stress field decays as $\sigma \sim \sigma_{\text{appl}}(d/r)^2$, where σ_{appl} is the magnitude of the applied stress, d is the diameter of the spot, and r is the distance from the spot. Consequently, the characteristic length over which the stress field decays is the same as the size of the spot. Our models are consistent with the cellular tensegrity model [26,27]. However, a model of a homogeneous network of prestressed elements [28], like all homogeneous elastic models, does not predict the long-distance propagation of forces. By a "locally applied force" we mean that the spot over which the force is applied is much smaller than the cell; for example, the spot can be a focal adhesion. By "long-distance propagation" we mean that the distance over which a force propagates is much larger than the size of the spot over which the force is applied. When a micropipette pulls an entire cell or a large portion of the cell, the force is not locally applied; such experiments do not test the notion of action at a distance.

We have focused on the propagation of forces along actin bundles, which have been observed in many cell types in culture and in vivo [29,30]. The basic principles of our models, however, are applicable to any prestressed, stiff bundles. For example, prestressed intermediate filaments may also guide forces over long distances; their absence compromises the ability of fibroblasts in stress transfer, cell migration, and cell growth [31,32]. As another example, microtubules are physically associated with actin bundles in migrating cells [33]; we speculate that microtubules, stiffened by intermediate filaments, together with actin bundles, may function as an integrated mechanical unit to guide forces over long distances. In mitotic cells, there is some evidence that forces might also propagate over long distances [4]. If so, our models suggest that the CSK should form conduits of some kind to guide forces over long distances. Whether or how such conduits form is unclear.

In contrast to signaling mediated by diffusion of soluble factors, signaling mediated by forces requires no transport of molecules and is *instant* over a long distance. A single actin bundle (or equivalently, a group of linked actin bundles) can span over the length of the cell. As the entire actin bundle moves, so do molecules in the CSK and cytosol near the actin bundle. Consequently, the message that a force is applied at one end of the bundle is *broadcast* to all the molecules near, and along the length of, the entire bundle. The instant, long-distance propagation of forces may as well be a strategy used by the cell to coordinate local activities at different parts of the cell.

Acknowledgments

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