A mechanical model of actin stress fiber formation and substrate elasticity sensing in adherent cells

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Tissue cells sense and respond to the stiffness of the surface on which they adhere. Precisely how cells sense surface stiffness remains an open question, though various biochemical pathways are critical for a proper stiffness response. Here, based on a simple mechanochemical model of biological friction, we propose a model for cell mechanosensation as opposed to previous more biochemically based models. Our model of adhesion complexes predicts that these cell-surface interactions provide a viscous drag that increases with the elastic modulus of the surface. The force-velocity relation of myosin II implies that myosin generates greater force when the adhesion complexes slide slowly. Then, using a simple cytoskeleton model, we show that an external force applied to the cytoskeleton causes actin filaments to aggregate and orient parallel to the direction of force application. The greater the external force, the faster this aggregation occurs. As the steady-state probability of forming these bundles reflects a balance between the time scale of bundle formation and destruction (because of actin turnover), more bundles are formed when the cytoskeleton time-scale is small (i.e., on stiff surfaces), in agreement with experiment. As these large bundles of actin, called stress fibers, appear preferentially on stiff surfaces, our mechanical model provides a mechanism for stress fiber formation and stiffness sensing in cells adhered to a compliant surface.

biological friction | cell biomechanics | cytoskeleton | focal adhesions | mechanosensitivity

All cells sense and respond to their environment. A prototypical example is chemical sensing mediated by cell-surface receptors, e.g., a neuron cell can sense small changes in external neural transmitter concentration and responds by opening ion channels and changing internal membrane polarization. A different kind of sensing has been receiving attention lately where some cells directly respond to mechanical properties of their environment, such as the stiffness of the surface to which they adhere. Understanding how these cells sense and respond to their mechanical environment and how they are able to translate mechanical cues into a chemical response are both topics of great interest in cell biomechanics.

Here, we are motivated by how stem cells sense and respond to their local mechanical environment. Viability of mesenchymal stem cells depends on their adherence to a surface. Interestingly, the differentiation of these cells is dependent on substrate stiffness. For example, stem cells on soft surfaces become primarily brain cells; on intermediate surfaces they become muscle cells and on stiff surfaces they become bone (1). Precisely how these cells sense surface stiffness, and how surface mechanics leads to the underlying biochemical changes that determine cell identity are still open questions (2). Several processes are thought to be involved. In particular, adhesion complexes (ACs), nonmuscle myosin II, and stress fibers are all thought to play a role. In fact, it seems likely that these three processes are interdependent (3).

Cells on surfaces are not static. Instead, they are constantly extending and retracting protrusions (lamellipodia, large sheet-like protrusions, and filopodia, thin finger-like protrusions) (5–8). The growth of these protrusions is thought to be driven by grow-

ing networks of actin filaments (see Fig. 1). Underneath these protrusions are adhesions with the substrate, clusters of membrane bound proteins (e.g., integrins) that bind to the surface beneath the cell. On stiff surfaces, these adhesions mature into large, stable protein complexes up to a few microns across, called focal adhesions. Conversely, on soft surfaces, these adhesions remain small and dynamic, called ACs. These adhesions translocate from the cell periphery toward its center in a size-dependent manner, with smaller adhesions moving at about 20 $\mu\text{m/hr}$ and larger ones more slowly (10). AC formation and dynamics are key features underlying cell mechanosensitivity (9).

As illustrated in Fig. 1, retraction of actin protrusions and other force generation between the cellular environment and the cytoskeleton are thought to be driven by nonmuscle myosin II (10–12). This protein belongs to the same group of proteins that underlie muscle contraction, having two heads each capable of hydrolyzing ATP and turning chemical energy into force and/or mechanical work (13). When nonmuscle myosin II's activity is inhibited, the cell's final functional determination (e.g., neural, muscle, or bone) is independent of surface stiffness, suggesting that the cells have lost their mechanosensing ability (1).

The cytoskeletal organization of adhered cells also depends on the substrate stiffness (see Fig. 1). Cells on soft surfaces have a diffuse cytoskeleton, composed of a near random arrangement of actin filaments. In contrast, cells on stiff surfaces contain many stress fibers, aggregations of actin, and other proteins that slowly contract under the influence of nonmuscle myosin II (14). Additionally, the inhibition of cross-linking proteins known to favor actin aggregation, such as α -actinin, leads to deficits in adhesion complex/focal adhesion formation and stiffness sensing (9).

Here, we create mechanically based models for each of these systems and show how they work together (see Fig. 2). We use a simple mechanical model that assumes ACs consist of a population of proteins (e.g., integrins) that bind to and unbind from a surface of extracellular matrix (ECM) molecules. We show that mature ACs have a simple constitutive law relating applied force and sliding rate. In particular, the AC provides a linear viscous drag, with a drag coefficient that depends on attachment rate, detachment rate, and the stiffness of the surface. Next we propose that nonmuscle myosin II behaves, at least qualitatively, like muscle myosin II and has a steady-state force-velocity relation that is well described by Hill's force-velocity relation (15). Therefore, we predict that a particular AC moving under the influence of nonmuscle myosin II will move faster and experience less tension on a soft surface than on a stiff one. Finally, we propose a simplified model for the actin cytoskeleton. We model each actin filament as a rigid rod of identical length constrained to move in two dimensions. Proteins (e.g., α -actinin) anchored along the length of the

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Fig. 1. A cartoon of stiffness sensing in cells. Left is a schematic diagram of a cell on a soft surface, right is a diagram of a cell on a stiff surface (based on figure 1b of ref. 4), where the soft surface has a Young's modulus of 10 kPa and the stiff surface a modulus of 100 kPa. Adhesion complexes are shown as black dots, stress fibers as thick gray lines. The magnified region, far right, shows how the cell interacts with the surface (the size scale, *Black Line*, is about 5 μm). Either filipodia, thin projections of actin bundles, or the lamellipodia, a flat, broad expanse of actin, extend via actin polymerization. Connections between the end of these actin filaments appear (adhesion complexes) and the cell pulls on them, through the force-generating properties of nonmuscle myosin II. In some cases, and more frequently on stiff surfaces, the adhesion complexes increase in size and become elongated in the direction of force, and the actin forms a bundle that eventually becomes a stress fiber.

actin filaments can bind to adjacent filaments. Like the integrins in the ACs, these actin cross-linking proteins provide a linear viscous drag (both in linear and rotational movements). This drag depends on the relative angle between the two filaments. When a constant force is applied to a random network of these filaments over a long time, the filaments align with the force and bunch together. We argue that this process is important in stress fiber formation and stiffness sensing.

When we put these three simplified models together, we find that random actin networks on stiff surfaces bundle together and orient along the line of applied force. Conversely, random actin networks on soft surfaces tend to remain random. We conclude that the sensing of surface stiffness as well as the formation of stress fibers can be explained through the interaction of passive mechanical systems with myosin as opposed to chemical signaling pathways, although these signaling pathways can enhance, stabilize, and regulate these mechanical effects.

Models

Biological friction. We first consider the problem of two surfaces or filaments that slide slowly relative to each other while proteins (or other long-chain molecules) form transient attachments between them. We develop analytical estimates of force-velocity behavior in this problem. In subsequent sections, we argue that this general problem can be applied to ACs and the actin cytoskeleton. This problem is discussed in more detail in *SI Text*.

We assume that proteins are anchored rigidly on one surface and interact with binding sites on the other surface. We model these proteins as point masses on zero-length linear springs. Using Kramers' theory, simple expressions for the detachment rate as a function of strain $k_d(x)$ and the attachment rate probability density $\rho_a(x)$ may be found (see (16, 17) and *SI Text*).

Assuming that the density of surface proteins is large, the following differential equation relates the binding probability distribution $n(x, t)$ to sliding rate (v) (18, 19):

$$\frac{\partial n}{\partial t} + v \frac{\partial n}{\partial x} = (1 - N_b)\rho_a - k_d n$$

where to first order, $n(x, t)dx$ is the probability of finding a surface protein attached with strain between x and $x + dx$ at time t . N_b is the proportion of bound cross-bridges, $N_b = \int_{-\infty}^{\infty} n dx$. Using the simple expressions for ρ_a and k_d from Kramers' theory, we can find analytic solutions to this equation. In particular, at small sliding rates, we may write the force per molecule as (17) and *SI Text*:

$$F = \kappa \frac{k_a}{k_d^0} \left(\frac{1}{k_d^0 + k_a} \right) v \quad [1]$$

Where k_d^0 is the detachment rate at zero strain, $k_a = \int_{-\infty}^{\infty} \rho_a(x) dx$ is the overall attachment rate, and κ is the stiffness of the molecular spring. This result will be used throughout to simplify the systems considered.

A Model for Adhesion Complexes. At the interface of ACs, integrins interact with proteins or other ECM molecules fixed to the surface (see Fig. 2). We assume that each of these proteins functions approximately like a linear spring with spring constant κ_f (proteins on the adhesion complex) and κ_s (proteins on the surface).

The stiffness of a protein on the surface comes from two springs in series: One spring represents the protein's intrinsic stiffness κ_p , and the other represents the stiffness of the surface κ_c . Assuming that the protein applies a force to the surface uniformly across a circle of area R , and assuming that the surface is linear elastic, uniform, isotropic and incompressible, we can write an expression that relates κ_c to the material properties of the surface (i.e., its Young's modulus E). Using this expression and defining a "series protein stiffness" $\bar{\kappa} = \kappa_f \kappa_p / (\kappa_f + \kappa_p)$, we can write the analogous expression for the friction generated by an ensemble of N_{ac} molecules at small sliding rates (Eq. 1 and see the justification of slow sliding rates for ACs in *SI Text*).

$$F_{ac} = N_{ac} \bar{\kappa} \frac{2\pi RE}{2\pi RE + 3\bar{\kappa} k_d^0} \frac{k_a}{k_d^0} \left(\frac{1}{k_d^0 + k_a} \right) v$$

In obtaining this expression (presented in detail in *SI Text*), we assume that molecules do not compete for binding partners and that the average spacing between proteins is small compared to $\sqrt{k_B T / \bar{\kappa}}$ (see the discussion of the dense binding site limit in refs. 16 and 17).

Note that this expression is simply linear viscous drag with a drag constant that depends on the stiffness of various proteins, the rate constants for attachment and detachment (in the absence of load), and surface stiffness (E). The drag coefficient is

$$b_{ac} = N_{ac} \bar{\kappa} \frac{2\pi RE}{2\pi RE + 3\bar{\kappa} k_d^0} \frac{k_a}{k_d^0} \left(\frac{1}{k_d^0 + k_a} \right) \quad [2]$$

Myosin As a Sensor of Surface Stiffness. Mature ACs move toward the cell nucleus because of the action of nonmuscle myosin II. Further, these motors work on one side against a more-or-less static actin cytoskeleton and on the other against an AC (see Fig. 2). Thus, depending on the force applied by the motors, the AC will slide at a different rate. However, molecular motors, including nonmuscle myosin II, typically have kinetics that depend on external load (20) and consequently the force they produce depends on their rate of travel. Thus, to understand how cells sense surface stiffness, we must understand the relationship between sliding rate (velocity) and force for nonmuscle myosin II.

Muscle myosin II has a force-velocity relation, even at the level of a few molecules (21–23), of the form:

$$\frac{F_{\text{myo}}}{F_0} = \frac{v_0 - v}{v_0 + cv} \quad [3]$$

first proposed by A.V. Hill in 1938 (15). Nonmuscle myosin II likely has a similar force-velocity relation. We now assume this relationship, with free parameters c , F_0 , and v_0 . In conjunction with our model for AC sliding over an elastic surface, this force-velocity relation allows myosin to sense surface stiffness.

Fig. 2. A schematic diagram of a cell, showing magnified views of an adhesion complex (AC), nonmuscle myosin II and the cytoskeleton. Nonmuscle myosin II generates force between antiparallel actin filament bundles, one of which is anchored on an AC, the other interacts with the actin cytoskeleton. In both the AC and the cytoskeleton, proteins are drawn as masses on springs in order to indicate how they function in our model.

Actin Cytoskeleton. A number of cytoskeleton models have been published, many of which idealize the cytoskeleton as a network of elastic rods connected by rigid nodes. As load is applied, the system is assumed to be in mechanical equilibrium (e.g., 24, 25). Such models are useful in understanding short time-scale behavior of the cytoskeleton. Here, however, we are interested in long time-scale reorganization of the cytoskeleton that involves numerous binding/unbinding cycles of the actin-binding proteins. Therefore, here we introduce a previously undescribed model for the cytoskeleton that incorporates the mechano-chemistry of these actin-binding proteins, allowing for the long time-scale reorganizations necessary for stress fiber formation.

We idealize the cytoskeleton as a large number of identical, rigid filaments randomly oriented in 2D. Because the cytoskeleton is not static but rather constantly being broken down and rebuilt, there is some time scale over which the cytoskeleton reforms. We model this time scale by removing filaments at some rate k_{to} and immediately replacing them with a random orientation and position (keeping the filament density constant). In order to model the effect of filaments anchored to the membrane, organelles, or the surface (and to stop the whole cytoskeleton from accelerating when force is applied) we fix a small number of these filaments on frictionless hinges.

We assume that AC-associated bundles of actin filaments are evenly distributed through this large 2D “sea” of actin and that the density of these bundles is constant with time. Myosin associated with these bundles applies a load to the cytoskeleton. As simulating a large 2D sea of actin is unfeasible, we simulate a smaller region with periodic boundary conditions and apply load to a single filament (see [SI Text](#) for further discussion of the boundary conditions), thus ensuring constant densities of free, fixed, and AC-associated actin.

We use this cytoskeleton model to understand how external loads affect the orientational distribution of actin filaments in the cell. For simplicity, we neglect the Brownian motion of the actin filaments (see *Discussion and Conclusions*). Neglecting random fluctuations, a filament experiences three different types of forces and/or torques. First, there may be an external force on the filament. Second, there is some (small) viscous force that occurs as each filament moves linearly or spins in the fluid. We neglect this force. Third, intersecting filaments interact with each other due to the interactions of proteins that bind one to the other. For simplicity, we use a continuum approximation, assuming that these actin-binding proteins are relatively numerous and firmly anchored onto one or the other actin filament. Then, using our biological friction model (Eq. 1, see *SI Text* for justification of the slow sliding limit for actin) and various geometrical arguments, the equations of motion of these filaments (here we consider the i th filament) are:

$$\sum_j \mathbf{F}_{ij} + \mathbf{F}_{\text{ext}}^i = 0 \quad \sum_j T_{/g}^{ij} = 0 \quad [4]$$

where $\mathbf{F}_{\text{ext}}^i$ is the external force applied to filament i and $\mathbf{F}_{ij}$ and T_{ij}^g are, respectively, the friction and torque about the center of mass applied on filament i by the relative sliding of filament j . In addition to these $3N_f$ differential equations that must be numerically integrated to find the cytoskeleton's time-dependent reorganization under load, where N_f is the total number of actin filaments, we add $2N_c$ constraint differential equations, where N_c is the number of fixed actin filaments.

Note that both F_{ij} and $T_{/g}^{ij}$ depend on a friction parameter that is defined as follows:

$$b_{\text{act}} = N_{\text{act}} \frac{\hat{\kappa}_s \hat{\kappa}_f}{\hat{\kappa}_s + \hat{\kappa}_f} \frac{\hat{k}_a}{\hat{k}_d^0 \hat{k}_a + \hat{k}_d^0} \quad [5]$$

and nondimensional functions that relate overlap area and the relative angle between filaments (*SI Text*). Note that $\hat{k}_s$ and $\hat{k}_f$ are the stiffnesses of the actin-binding proteins and their binding sites, respectively. The rate constants $\hat{k}_a$ and $\hat{k}_d^0$ are the attachment and detachment rates of the proteins in the absence of strain.

Results

The Interaction of Adhesion Complexes with Myosin Results in a Stiffness-Dependent Force. We assume that the cytoskeleton is stiff. Thus, as myosin applies a force between an AC and the cytoskeleton, the sliding rate of the AC is equal to the shortening rate of myosin (note that the following results also hold without this stiff cytoskeleton assumption, as discussed in *SI Text*). Because the force on the AC (F_{ac}) is the same as the force generated by myosin (F_{myo}), using Eqs. 2 and 3 this assumption allows us to relate the stiffness of the surface to the magnitude of the force that myosin applies to the cytoskeleton ($|F_{ext}|$):

$$|\mathbf{F}_{\text{ext}}| = F_{\text{myo}} = F_0 \frac{v_0 - v}{v_0 + cv} = F_{\text{ac}} = b_{\text{ac}}(E)v$$

We can solve this equation directly for myosin force as a function of surface stiffness.

In general, we find that at low surface stiffness, ACs slide quickly and myosin generates only small forces. At high stiffness, ACs slide slowly and myosin generates larger forces. Thus, the interaction of myosin and the ACs leads to differential forces depending on surface stiffness.

The Time Scale of the Cytoskeleton. In the absence of actin turnover ($k_{to} = 0$), the cytoskeleton has an important time scale

$$\tau = \frac{b_{\text{act}} L}{|F_{\text{ext}}|}$$

where b_{act} is defined in Eq. 5, F_{ext} is the applied load, and L is the length of the filaments. If we nondimensionalize using this time scale, the equations of motion (Eq. 4) become

$$\sum_j \mathcal{F}_{ij} + \sum_k \delta_{ik} e_f^i = 0 \quad \sum_j \mathcal{T}_{ij}^j = 0 \quad [6]$$

where $\mathcal{F}_{ij}$ are the nondimensional friction forces and $\mathcal{T}_{ij}^j$ are the torques about the center of mass. The constraint equations and constraint forces may be similarly nondimensionalized.

Note that these equations depend only on a single nondimensional parameter, the ratio of the filaments' width (h) to their length (L): $R_L = h/L$. We assume this value is around 0.01. We arrive at this value by assuming that the width of an actin filament is on the order of 10 nm, while its length is on the order of 1 μm . These are order-of-magnitude estimates, so our estimate of R_L is also roughly correct for bundles of actin as well. For a system with given aspect ratio and initial distribution, varying either applied force or drag coefficient simply changes the time scale of the dynamics rather than the details of the dynamics themselves.

In conjunction with the result from the previous section, where on stiff surfaces myosin generates a larger force than on a soft surface, we may use the cytoskeleton time scale to understand how surface stiffness affects cytoskeleton reorganization. In particular, because myosin is responsible for the external force on the cytoskeleton (F_{ext}), the cytoskeleton time scale is smaller on a stiff surface than on a soft one. In the next section, we examine the dynamics of stress fiber formation in our cytoskeleton model and show that the interaction of the actin-turnover time scale and the cytoskeleton time scale leads to a mechanism for surface stiffness-dependent stress fiber formation.

Stress Fiber Formation in the Cytoskeleton Model. We performed a series of numerical simulations with our cytoskeleton model. In these simulations, 150 actin filaments are oriented randomly in 2D. We assume periodic boundary conditions, with period $2.5L$, in order to minimize edge effects and to keep filament density constant (see *SI Text* for a more detailed discussion of boundary conditions). We apply a constant force in the positive y direction on a single filament and fix three filaments (see Fig. 3A). We investigate four different actin turnover rates, $k_{10}\tau = 0$, $k_{10}\tau = 0.0017$, $k_{10}\tau = 0.0033$, and $k_{10}\tau = 0.033$. In the simulations without actin turnover ($k_{10}\tau = 0$), different values of F_{ext} and b_{act} are examined in order to test our time-scale predictions. We chose these values so that one set of simulations would have a time scale three times shorter than the other set of simulations. These results are shown in Fig. 3C. The agreement is good.

Two results of these simulations are particularly noteworthy. First, as the simulations progressed, the filaments clumped together and became oriented parallel to the direction of the force (see Fig. 3B and C). These aggregations are an indication of stress fiber formation. Second, as actin-turnover rate increased, the size of these aggregations at steady-state decreased (see Fig. 3D).

When we apply force to a single filament, it moves through the other randomly oriented filaments. After a sufficient time, long enough for the filament under load to pass through the unloaded filaments several times owing to the periodic boundary conditions, large aggregations, sometimes of 50 filaments (one third of the total filaments) appear. Four frames of such a simulation are shown in Fig. 3B (see *Movie S1*). We may quantify this ordering of the cytoskeleton by defining a stress fiber of size N as being N or more filaments all of which have orientations within ε_θ of the direction of the applied force and have centers of mass coordinates orthogonal to the direction of force application that are all clustered within some distance ε_x . For our simulations, we used $\varepsilon_\theta = 0.1$ (about 6°) and $\varepsilon_x = 0.2L$ to identify stress fibers. When

Fig. 3. Results of cytoskeleton model simulation. (A) The cytoskeleton model. A constant force is applied in the y direction on a single filament (Black). Three filaments (Dark Gray) are fixed in space with frictionless hinges at one end. Periodic boundary conditions are assumed. (B) Four snapshots from a simulation (full movie is shown in *SI Text*). As the simulation progresses, a large aggregation of actin filaments appears, oriented in the direction of the applied force (Arrow). (C) Time course of stress fiber formation probability (n_{sf}) in the absence of actin turnover $k_{10} = 0$. Left, demonstrating the time scale of the cytoskeleton. Inset shows stress fiber formation probability for an aggregation of 10 actin filaments as a function of time for two different choices of applied force, filament length, and actin friction parameters. When time is nondimensionalized with the cytoskeleton time scale τ , the two curves fall along a single line. Right, actin aggregations of various sizes show similar formation dynamics. When time is rescaled, the probability of formation of actin aggregations of various size fall along a single line. Thus, we expect that bundles of arbitrary size will be eventually be formed, given enough simulation time. (D) Top, time course of stress fiber formation probability in the presence of actin turnover ($k_{10}\tau = 0.0017$ not shown). As actin turnover rate increases, the steady-state probability of bundle formation (n_{sf}^{∞}) decreases, indicating that there is a balance between stress fiber formation and breakdown. Bottom, steady-state stress fiber probability as a function of bundle size for four different values of actin turnover.

Fig. 4. Stress fiber formation as a function of surface stiffness. By choosing parameters in our model, we calculate the steady-state probability of stress fiber formation (n_{sf}^{∞}) of various sizes (N) as a function of the Young's modulus of the surface. At low stiffness, no stress fibers form, while at high stiffnesses many, large stress fibers form. Inset, simulation results for stress fibers of 10 actin filaments (Gray, $\pm$ sd) and an interpolation between the results (Black). Details of the interpolation are in [SI Text](#).

certainly change the detailed behavior of the simulations, they will not change the qualitative results. For example, Brownian motion would tend to erase the memory of the system over long time scales, serving the same role as actin turnover in our model. Adding these effects would require a different modeling approach than the one presented here, for example a continuum approach (e.g., refs. 31–33), though such simulations would still represent a significant computational challenge.

A Mechanical Picture for Stiffness Sensing. Although each individual model is simple, the overall behavior of the models captures the emerging principles in this system. ACs directly interact with the surface, providing a viscous drag that increases with elastic modulus of the surface. As a consequence of its steady-state force-velocity relation, myosin II, sliding actin filaments fixed to a more-or-less rigid cytoskeleton on one side and the sliding AC on the other, generates greater forces the slower the ACs slide. As external force is applied to the cytoskeleton, actin filaments aggregate and orient parallel to the direction of force application, indicating stress fiber formation. The greater the external force, the faster this aggregation occurs. Because the steady-state probability of forming a stress fiber reflects a balance between the time scale of stress fiber formation and the time scale of stress fiber destruction (because of actin turnover), more stress fibers are formed when the cytoskeleton time scale is small, i.e., on stiff surfaces. By choosing values for the various parameters in our model, we may plot the probability of stress fiber formation as a function of surface modulus (Fig. 4). As expected, at low stiffness no stress fibers form; while at high stiffness, many stress fibers form. Thus, the stiffness of the substrate directly influences the relative timescales of processes in the cytoskeleton, leading to differential formation of stress fibers and ultimately leading to the changes in cellular biochemistry that underlie cell lineage specification in stem cells.

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