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Formation of contractile networks and fibers in the medial cell cortex through myosin-II turnover, contraction, and stress-stabilization

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Abstract

The morphology of adhered cells depends crucially on the formation of a contractile meshwork of parallel and cross-linked fibers along the contacting surface. The motor activity and minifilament assembly of non-muscle myosin-II is an important component of cortical cytoskeletal remodeling during mechanosensing. We used experiments and computational modeling to study cortical myosin-II dynamics in adhered cells. Confocal microscopy was used to image the medial cell cortex of HeLa cells stably expressing myosin regulatory light chain tagged with GFP (MRLC-GFP). The distribution of MRLC-GFP fibers and focal adhesions was classified into three types of network morphologies. Time-lapse movies show: myosin foci appearance and disappearance; aligning and contraction; stabilization upon alignment. Addition of blebbistatin, which perturbs myosin motor activity, leads to a reorganization of the cortical networks and to a reduction of contractile motions. We quantified the kinetics of contraction, disassembly and reassembly of myosin networks using spatio-temporal image correlation spectroscopy (STICS). Coarse-grained numerical simulations include bipolar minifilaments that contract and align through specified interactions as basic elements. After assuming that minifilament turnover decreases with increasing contractile stress, the simulations reproduce stress-dependent fiber formation in between focal adhesions above a threshold myosin concentration. The STICS correlation function in simulations matches the function measured in experiments. This study provides a framework to help interpret how different cortical myosin remodeling kinetics may contribute to different cell shape and rigidity depending on substrate stiffness.

Keywords

Myosin; Cell Cortex; Stress Fibers; Mathematical Modeling; Image Correlation Spectroscopy

Introduction

The ability of cells to adhere and exert forces on substrates of different chemical and mechanical composition relies on their ability to form adhesions and transmit signals to the cytoskeleton [Schwarz and Safran 2013]. The actin filaments and myosin motors in the cell cortex connect to focal adhesions and adjust to form networks below the plasma membrane that maintain tension and traction. This in turn determines cell shape and mechanical rigidity [Discher et al. 2005; Shutova et al. 2012; Yeung et al. 2005].

Bundled actomyosin structures that can anchor at focal adhesions and at the cell nucleus are important subcellular structures for generating cell traction and adhesion [Vicente-Manzanares et al. 2009]. One such type are stress fibers, which are bundles of actin filaments containing myosin-II motors and α -actinin cross-linking proteins. They can form through different pathways and can have different orientation with respect to the cell axis as well as varying distribution of actin filament polarity [Burridge and Wittchen 2013; Das et al. 2012; Naumanen et al. 2008; Vallenius 2013]. Stress fibers can form from actin filaments polymerized at cell protrusions: in motile cells, actin filaments nucleated at lamellipodia and filopodia undergo retrograde flow and are pulled together by myosin motors into bundles such as transverse actin-arcs and dorsal stress fibers [Burridge and Wittchen 2013; Naumanen et al. 2008]. Cortical actomyosin fibers can also form at the cell cortex away from leading edge protrusions (or away from the contractile back for the case of cells using bleb-based motility). It was recently shown that the medial cortex of adhered cells consists of dynamic formin, actin, and myosin-II foci that contract and coalesce into clumps when actin filaments are depolymerized with Latrunculin A (LatA) [Luo et al. 2013b; Rossier et al. 2010]. Constant turnover of the foci was found to be necessary for the observed patterns [Luo et al. 2013b].

The animal cell cortex is able to reorganize into different dynamic steady states upon mechanical or pharmacological perturbations, which reflects its inherent plasticity. Such morphological changes are also seen in simpler organisms such as fission yeast where the mitotic medial actin network can organize into different morphologies such as clumps, contractile rings, and extended meshworks [Laporte et al. 2012]. In vitro experiments reconstituting actomyosin structures from a set of purified components also demonstrate this cytoskeletal plasticity [Alvarado et al. 2013; Kohler et al. 2011].

In this paper we study live cells and present experiments and simulations that explore the mechanisms of cortical plasticity and response to perturbations. For the case of adherent cells used in this study, this perturbation is the linking of the cytoskeleton to a substrate through focal adhesions. In addition to turnover and contraction, we anticipate an important role of mechanical forces and tension since the cortical actin and myosin concentrations respond to both external and internal forces. For example, myosin-II and cross-linkers accumulate at sites of micropipette aspiration in a manner that depends on actin filament cross-linker concentration [Luo et al. 2013a; Ren et al. 2009].

The precise kinetics of cortical cytoskeletal dynamics depend on many proteins and signaling pathways [Burridge and Wittchen 2013]. Here we do not attempt to address the

precise molecular regulation but instead look at the collective behavior of myosin-II distribution. Using statistical analysis of our data we outline the basic mechanisms that should combine to generate the observed patterns. We also do not try to describe the full pathway leading to stress fiber formation, which may involve a cascade of stabilization processes, but rather describe the mechanisms that provide the initial geometric and mechanical properties necessary to stabilize actomyosin structures. To address this question experimentally we used confocal microscopy to study HeLa cells stably expressing MRLC-GFP (that binds to myosin-II) adhered to a glass slide. Numerical simulations developed based on our measurements provide support for our proposed underlying mechanisms.

Results

Types of medial cortical myosin networks in adhered HeLa cells

We performed experiments to quantify the distribution of myosin, actin and focal adhesions at the cell cortex of adhered HeLa cells, see Fig. 1. We used HeLa cells stably expressing MRLC-GFP [Kamijo et al. 2006] cultured on cover glass in regular DMEM medium for three days. Cells were then fixed and stained for either F-actin with rhodamine-phalloidin or focal adhesions with a vinculin antibody (see Materials and Methods). Confocal microscopy images were obtained by focusing on the adhered part of the cell cortex. Actin and myosin formed stress fibers that end at focal adhesions at the periphery of almost all cells. However the morphology of the actomyosin network in the central (medial) region on the basal plane of the cells adjacent to the slide varied from cell to cell. We classified the observed structures into three types (see Fig. 1A and B). Type I cells (70%) have filament bundles of length comparable to the cell size; these fibers connect focal adhesions located at the boundary or middle cell region. In Type II cells (20%), medial myosin and actin form short fibers and networks partly anchored by focal adhesions located at the boundary or middle cellular region. Type III cells (10%) have some MRLC-GFP intensity in the medial region but there are no detectable fibers or networks and have a very small number of medial focal adhesions (less than 3 per cell). The medial structures of Type III cells resemble the less organized MRLC-GFP distribution nearby the fiber/network structures of Type I and II cells.

We performed further analysis to compare different cell types. The area of the adhered part of the cell is similar in all cell types (Fig. S1B). The average MRLC-GFP intensity over the whole cell is generally less for Type III cells (Fig. S1D-F). However because this number may depend on the expression level of MRLC-GFP, we also calculated intensity ratios after imaging a single confocal slice focused on the adhered part of the cell. We found that the ratio of average MRLC-GFP intensity in the cell middle (the part of the cell that excludes the peripheral stress fibers) over the average of MRLC-GFP intensity on the whole cell is significantly less in Type III cells compared to Type I and II (Fig. 1C).

We also measured the number of focal adhesions in the cell middle and over the whole cell for all three cell types (see Fig. 1D, Materials and Methods, and Fig. S2). Type I cells have more focal adhesions compared to Type III cells (both total and in cell middle). We did not find a statistically-significant difference between the total number of focal adhesions in Type I and II cells, however we note that the number of peripheral focal adhesions in Type I

cells may be slightly underestimated since it is difficult to isolate and distinguish the focal adhesions on the boundary of the cell (see Fig. S2). It is interesting to notice that Type II cells have more adhesions in the cell middle compared to the other two cell Types. The density of focal adhesions in the middle of Type I, II and III cells are 1.4 ± 1.1 , 3.9 ± 2.4 and 0.3 ± 0.06 per $100 \mu\text{m}^2$ (Mean $\pm$ StDev), respectively.

The above analysis shows that all cell types recruit myosin in the medial cortex. It appears that the ability of cells to form medial fibers and to tune their morphology is correlated with their ability to form focal adhesions in the cell middle. To better understand how different medial myosin distributions are generated, we turned to time-lapse imaging of cells expressing MRLC-GFP.

Dynamics of medial cortical myosin in adhered HeLa cells

By imaging a single confocal slice focused at the adherent part of the cell, we observed that peripheral stress fibers labeled by MRLC-GFP maintain their position and shape over 30 min. The medial part of the cell cortex labeled by MRLC-GFP is however very dynamic over the same timescale (Fig. 2 and Movies 1-3).

Fig. 2A (Movie 1) shows a Type I cell in which MRLC-GFP foci appear and disappear around more stable peripheral and medial fibers. During this turnover process, the foci align to form transient fibers over $\sim 5 \mu\text{m}$ (see panel at 6:00 min in Fig. 2A). The foci turnover process is also seen in the volume kymograph in the first panel of Fig. 1D, which is constructed from cell region of the panels in Fig. 2A. In this kymograph the transient MRLC-GFP foci appear as bright spots.

Fig. 2B (Movie 2) shows a Type II cell where MRLC-GFP foci turnover and transient alignment is seen in the panels showing an enlarged region of cell cortex. This turnover is also seen in the transient spots in the volume kymograph in the middle panel of Fig. 2D. The kymograph highlights another process: directed foci motion. Foci motion is evidently a result of contraction and the likely origin of transient clumping (see panel at 1:40 in Fig. 2B). Similar dynamics to the Type II cell of Fig. 2B are observed in the cells of Fig. 2C, which we would classify as Type III, and the corresponding volume kymograph in the last panel in Fig. 2D.

Taken together, Figs. 1 and 2 indicate that the process of medial cortical fiber formation results from myosin foci assembly and disassembly at the cell cortex (likely in the form of minifilaments on the cell cortex [Shutova et al. 2012; Verkhovsky et al. 1995]), alignment (likely through pulling on actin filaments nucleated near the cell cortex) and stabilization of linear elements. Since similar dynamics are observed in all three cell types, the different cortical morphologies observed seem correlated with the ability of cells to form fibers anchored at focal adhesions in the medial part of the cell cortex. These results also highlight a pathway to actomyosin fiber formation different to that of more motile cells. In many motile cells peripheral arcs and fibers form through myosin-mediated collapse of peripheral lamellipodial protrusions [Aratyn-Schaus et al. 2011; Burnette et al. 2011; Naumanen et al. 2008; Tojkander et al. 2011]. By contrast, the fiber pattern of HeLa cells on glass slides is

dependent on the steady state properties of the medial cell cortex. In some cells such as Fig. 2B, an outward rather than inward flow can be observed.

STICS analysis of cortical MRLC-GFP in adhered HeLa cells

To quantify medial MRLC-GFP dynamics, we used spatio-temporal image correlation spectroscopy (STICS) [Kolin and Wiseman 2007]. We used this method since the density and complexity of foci motions precluded methods such as single-particle tracking, even with manual editing [Smith et al. 2011]. STICS allows measurement of correlations of intensity over space and time, which provides information on the diffusive, transport, and turnover kinetics of the fluorescently-labeled molecules [Kolin and Wiseman 2007; Wiseman and Petersen 1999; Yam et al. 2007]. It is a useful tool in images such as those of Fig. 2 in which intensity correlations persist from frame to frame but the motion of individual myosin foci cannot be tracked with good accuracy. The STICS function of relative distances ζ and η (along the x and y directions) and relative time τ is:

$$r(\zeta, \eta, \tau) = \frac{\langle \delta I(x, y, t) \delta I(x + \zeta, y + \eta, t + \tau) \rangle}{\bar{I}(t) \bar{I}(t + \tau)} \quad (1)$$

where $I(x, y, t)$ is intensity at pixels x, y of an $N \times N$ pixels image at frame corresponding to time t , $\bar{I}(t)$ is the average intensity at time t and $\delta I(x, y, t) = I(x, y, t) - \bar{I}(t)$. The $\langle \rangle$ average in Eq. (1) is an average over x, y and t .

We calculated $r(\zeta, \eta, \tau)$ by selecting regions of 100×100 pixels ($6.2 \times 6.2 \mu\text{m}$) in movies of cells expressing MRLC-GFP imaged every 10 s at a single confocal slice focused on the adhered part of the cell cortex, see Fig. 3A. The total number of frames was 16, which was sufficiently long to observe the decay in correlations over time. Since the focus of this analysis was to quantify the dynamic parts of the cell cortex, we used the following criteria to select regions for STICS: (1) The average intensity does not change by more than 2%, since we did not want to measure correlations due to net accumulation or disassembly; (2) The region does not contain permanent fibers or other intense spots that dominate the correlations; (3) The region does not exhibit net cortical flow, since we are interested in the dynamics at the rest frame of the $6.2 \times 6.2 \mu\text{m}$ cortical patch; (4) we did not include regions that lack MRLC-GFP foci. Panels in Fig. 2B, 2C and 3A are examples of regions used in the analysis. Because of the presence of medial fibers in Type I cells, all regions that fulfilled the above criteria were in cell Types II and III. We grouped data from Types II and III together in the following analysis.

The panels selected for STICS analysis are large enough to contain MRLC-GFP foci aligned in different directions so that, on average, there is no preferred orientation. Each selected region is wide enough and imaged for a long enough period such that the motions of the foci are along random directions, on average. Since we specifically excluded from our analysis regions that exhibit net displacement or large-scale asymmetric features, function $r(\zeta, \eta, \tau)$ is peaked at the origin for all τ (Fig. 3B). To simplify the analysis we thus performed an average over the polar angle to obtain a STICS function that depends on the distance to the origin $\rho = \sqrt{\zeta^2 + \eta^2}$:

$$r(\rho, \tau) = \frac{1}{2\pi\rho} \oint_{\rho} r(\xi, \eta, \tau) dl. \quad (2)$$

Fig. 3D shows $r(\rho, \tau)$ for the region in Fig. 3A, after removing the sharp peak at $r(0,0)$ due to white noise correlations [Wiseman and Petersen 1999] (see Materials and Methods). We observe that spatial correlations extend to distances longer than the microscope's resolution (about 0.2 μm) and persist at long times: the FWHM, defined here as twice the distance ρ at which $r(\rho/2, \tau) = r(0, \tau)/2$, is 0.65 μm at $\tau=0$. This is consistent with the organization of myosin-II into bipolar minifilaments of size 0.8-1 μm [Svitkina et al. 1997; Verkhovsky et al. 1995; Verkhovsky et al. 1999] and the alignment of these filaments along short fibers.

The function $r(\rho, \tau)$ decreases with increasing τ for a given ρ , which reflects loss of correlation due to either myosin assembly and disassembly at the cortex as well as movement due to local contractions. Fig. 3E shows that the decay of $r(0, \tau)$ can be fitted by a single exponential with decay time τ_{control} (Note: in the fit we exclude $r(0,0)$ which is determined by extrapolation, see Materials and Methods. Since $r(0,0)$ is less accurate compared to $r(0, \tau)$ for $\tau > 0$, it leads to noisy curves when used as the initial condition to normalize and compare different decay curves). We find that the average decay time is $\tau_{\text{control}} = 130 \pm 50\text{s}$ (Mean $\pm$ StDev, $n=21$), see Fig. 3F. This decay time reflects both the contraction of MRLC-GFP foci and their turnover. Both processes contribute to comparable amounts since the time for foci movement over a distance of order their size is comparable to their lifetime, see Fig. 2D.

To further probe how turnover and contraction contribute to cortical actomyosin organization we repeated the same analysis for cells treated with blebbistatin.

Changes of medial myosin structures in blebbistatin

To better understand how the activity of myosin II affects the process of actomyosin network assembly in the middle of HeLa cells, we conducted experiments with myosin-II inhibitor blebbistatin [Limouze et al. 2004] (see Materials and Methods). Fig. 4A shows cells expressing MRLC-GFP cultured with the same procedure as the control cells in Fig. 1 but treated with 100 μM blebbistatin for 60 min before fixing and staining for vinculin. The time of 60 min was chosen because it is long enough to observe a change in the fiber network, but not too long, since MRLC-GFP fibers disassemble completely after 2.5 hrs (Fig. S3). We observed a lower fraction of Type I cells and increased Type II and III as compared to control cells, reflecting the disassembly of myosin fibers. This indicates that myosin motor activity is important in the maintenance of long fibers. Fig. 4B shows results with cells treated with 100 μM blebbistatin for 60 min and then washed out by regular DMEM medium for 60 minutes before fixing and staining. The fraction of Type I cells increased to a value near that in control cells (Fig. 1), which indicates that MRLC-GFP fibers are restored by restoring myosin motor activity.

The number of focal adhesions found in the middle of Type II cells also decreases in the presence of blebbistatin (see Fig. S1A), indicating that focal adhesions are also stabilized by

stress, consistent with other studies [Balaban et al. 2001; Choquet et al. 1997; Lavelin et al. 2013; Shutova et al. 2012; Stricker et al. 2011]. Thus, blebbistatin perturbs the medial network in Type I and II cells that lose medial adhesions and some Type I cells become Type II or III; upon blebbistatin removal, a medial network of fibers can reform within 60 min but the overall number of medial adhesions does not recover to the control level. While the effect of blebbistatin on the cortical fibers HeLa cells is smaller compared to some cell types, such as U2O cells [Aratyn-Schaus et al. 2011; Hotulainen and Lappalainen 2006], the rate of loss of cortical MRLC-GFP in HeLa cells is comparable to a prior study in fibroblasts [Rossier et al. 2010], see Table SI.

We did not detect a statistically-significant change in the ratio of MRLC-GFP intensity in the cell middle over the whole cell by blebbistatin (see Fig. S1C). Interestingly, the absolute average MRLC-GFP intensity in the medial cortex in Type II and III cells is similar in control and blebbistatin-treated cells (see Fig. S1D-F). The medial MRLC-GFP intensity of Type I cells decreases in blebbistatin (see Fig. S1D), likely due to the disassembly of some stress-stabilized medial fibers in these cells.

We also noticed that total cell adhesion area decreases slightly when adding blebbistatin and increases again after blebbistatin washout (see Fig. S4). This trend is also seen in analysis of fixed cells in Fig. S1B and most notably for Type II cells that have smaller area (some cells, not included in Fig. S1B, lost adherence to the substrate and become round, see middle row of Fig. 4A). Loss and recovery of adherence in the medial cortex as well as in the peripheral stress fibers and protrusions likely accounts for this trend.

STICS analysis of cortical MRLC-GFP in adhered HeLa cells in blebbistatin

We quantified the dynamics and distribution of medial MRLC-GFP in cells treated with 50 μ M blebbistatin for 60 min, using the same method as in Fig. 3 by applying STICS to $6.2 \times 6.2 \mu$ m regions imaged every 10 s for 160 s, see Fig. 5A (Movie 4). The criteria for selecting regions were also unchanged. The 3D kymograph of Fig. 5B shows much less directed motions compared to untreated cells (Fig. 3D), consistent with the anticipated reduction in contractility. However individual MRLC-GFP foci continued to appear and disappear (note: in Fig. 5B we imaged for a shorter total interval compared to Fig. 3D to avoid laser-induced deactivation of blebbistatin [Kolega 2004; Sakamoto et al. 2005], see Materials and Methods and Fig. S5). The radially-averaged STICS function $r(\rho, \tau)$ has similar shape and width to untreated cells, however the decay of correlations with time is slower, see Fig. 5C. An exponential fit to $r(0, \tau)$ gives a relaxation time $\tau_{bleb} = 240 \pm 90$ s (Mean $\pm$ StDev), longer than that of untreated cells (see Fig. 3F).

Similar analysis to Fig. 5A-D was performed for experiments after blebbistatin washout. Cells were treated with 50 μ M blebbistatin for 60 minutes that was then washed out with regular DMEM medium for 60 minutes before imaging. An example of a cell is shown in Fig. 5E (Movie 5). Unlike cells in the presence of blebbistatin, contractile motions were now evident, see Fig. 5F. As a result of these motions, the decay of $r(0, t)$ in the cell of Fig. 5E occurs with decay time $\tau_{washout} = 123$ s, which is close to the decay time of untreated cells (see Fig. 3F). Even after the washout however, many cells are unable to recover and the resulting average decay time is only slightly smaller when compared to Fig. 5D: $\tau_{washout} =$

220±1602s (Mean ± StDev). The standard deviation of the decay time is larger compared to Figs. 3 and 5, reflecting a variability among cells that recovered and cells that were damaged with blebbistatin.

To further compare the different conditions in control, blebbistatin-treated, and recovered-from-blebbistatin cells (Figs. 3 and 5), we searched for correlations in STICS analysis data. We did not find significant correlation between average region intensity and decay time (Fig. S6A), between FWHM of $r(\rho,0)$ and decay time (Fig. S6B), and between $r(0,0)$ and decay time (Fig. S6C). The value of $r(0,0)$ is the magnitude of relative intensity fluctuations at a given frame (see Eq. (1)). We find that $r(0,0)$ is larger in the presence of blebbistatin (Fig. S6C), indicating a more inhomogeneous distribution in the selected regions. We did not detect a dependence of $r(0,0)$ on average intensity (Fig. S6D).

In conclusion, we have demonstrated that STICS analysis on the time-lapse MRLC-GFP images provides a tool to determine the activity of myosin II in live cells. The correlation decay time increases in blebbistatin, consistent with the visual observation of reduction in contractile myosin movements.

Model of myosin minifilament turnover and alignment

The results of the previous sections suggest that the medial actomyosin cortex exists in a state of assembly, reorganization and disassembly. We observed myosin foci appear and disappear over time, in a process that likely depends on myosin minifilament formation by nucleation and growth [Korn and Hammer 1988; Ren et al. 2009] and myosin binding to actin filaments and proteins on the cell membrane. The cortical foci contract towards one another and align. This process is likely to occur by myosin pulling on actin filaments located at the cell cortex, which tend to form bundles with myosin minifilaments and actin cross-linking proteins [Biro et al. 2013]. Formation of stable linear fibers depends on myosin motor activity and is positively correlated to presence of contraction, implying that the stability of myosin minifilaments in the cortex increases with tension, as also suggested by prior experiments [Luo et al. 2013a; Ren et al. 2009; Rossier et al. 2010; Shutova et al. 2012]. When coupled to adhesion formation, long fibers can thus develop along the cortex by maintaining tension through anchors at focal adhesions.

We developed a coarse-grained computational model to test the extent to which the above mechanisms can explain our experimental observations. In this model the basic units are bipolar myosin minifilaments that can move and change orientation on a 2D plane representing the cell cortex (see Fig. 6). They interact via the following four types of forces and torques they exert on one another.

1. Contractile force (Fig. 6A), mediated by actin filaments in the vicinity of the minifilaments (actin filaments are not explicitly included). The direction of the attractive force between minifilaments i and j is along the axis joining their centers and has magnitude

$$F_c^{ij} = k_c (s - L_{min}) \exp(-|\theta_{ij}|/\theta_c^0), \quad (L_{min} < s < L_{max}) \quad (3)$$

when the distance between the minifilament centers s is between $L_{\min}$ and $L_{\max}$ and zero otherwise. Here where k_c , $L_{\min}$, $L_{\max}$ and θ_c^0 are model parameters and $\theta_{ij} = \theta_1 + \theta_2$ where θ_1 and θ_2 are the angles between axis of each minifilament and the line joining their centers, as shown in Fig. 6A. The exponential dependence in Eq. (3) is an upper cut-off that simulates a decrease in contractile force when filaments lose alignment. After scanning values of θ_c^0 in the simulations, we chose $\theta_c^0 = 0.3$ (17.2°), which resulted in realistic myosin alignment. For simplicity, we chose a simple saw-tooth shape, as a function of s , that has a maximum at $L_{\max}$. However, our main results are independent of the precise shape of the contractile force versus distance function, as long as this is a function with an amplitude peaked between $L_{\max}$ and $L_{\min}$, see Fig. S7.

Parameters $L_{\min}$ and $L_{\max}$ are determined based on the size of myosin minifilaments and actin filaments. In human endothelial, epithelial, and fibroblast cells, the length of myosin II minifilaments is $0.6 - 2 \mu\text{m}$ and the length of actin filaments is $1 - 5 \mu\text{m}$ [Charras et al. 2006; Shutova et al. 2012; Svitkina et al. 1997; Thoresen et al. 2011; Verkhovsky et al. 1995]. So we chose $L_{\max} = 1.6 \mu\text{m}$ as the longest scale over which contraction occurs. When minifilaments approach one another they cannot overlap and accumulation of α -actinin in between them may limit how close they can approach one another. So we set $L_{\min} = 0.8 \mu\text{m}$ and apply a repulsive force for minifilament separation distances shorter than $L_{\min}$ (see below). The linear functional dependence on s in Eq. (3) ensures the contractile force vanishes continuously at $s = L_{\min}$.

Parameter k_c determines the magnitude of the contractile force. For non-muscle cells, there are about 20 myosin II motors polymerized into bipolar minifilaments in stress fibers [Langanger et al. 1986; Vicente-Manzanares et al. 2009]. Since each myosin motor produces 2-6 pN pulling force on actin filaments, a single minifilament could produce a pulling force ~ 40 pN, after considering the disordered nature of the actin network and the small duty ratio of myosin II heads [Erdmann and Schwarz 2012]. Thus we estimate $k_c = 50 \text{ pN}/\mu\text{m}$.

As minifilaments start to aggregate in the simulations, they can exert larger forces as they interact with more neighbors. To represent an upper physical limit to the magnitude of contractile forces, we introduced an upper limit of 50 pN per minifilament. For all minifilaments for which the total contractile force exceeds the upper limit, we reduce the force corresponding to every pair by the factor that reduces the total force to the maximal value. After performing this operation for all minifilaments, we then recalculate the average force between pair i and j to be $(F_c^{ij} + F_c^{ji}) / 2$.

2. Aligning force and torque (Fig. 6B) responsible for minifilament alignment and rotation. Since this effect likely occurs via actin filaments in between minifilaments, we apply these forces and torques when $s > L_{\max}$. Following [Ojic et al. 2011], aligning forces act on the centers of minifilaments, perpendicular to the line that joins their centers, see minifilaments i and j in Fig. 6B. The force on i that acts along the direction that decreases the magnitude of angle θ_1 defined in Fig. 6B. The unit vector along this direction is $\mathbf{e}_\perp^1$, and similarly for j that has angle θ_2 . To satisfy force balance, we assume a force of equal magnitude but of opposite

direction acting on the neighbor. We used the following expression for the force on minifilament i due to minifilament j :

$$\mathbf{F}_{al}^{ij} = F_{al} \left[\frac{\sin(\theta_1)}{C_{max}} e^{-\frac{|\theta_1|}{\theta_{al}^0}} \right] \mathbf{e}_\perp^1 - F_{al} \left[\frac{\sin(\theta_2)}{C_{max}} e^{-\frac{|\theta_2|}{\theta_{al}^0}} \right] \mathbf{e}_\perp^2 \quad (s < L_{max}) \quad (4)$$

Parameter F_{al} determines the magnitude of the aligning force. Here $C_{max} = 0.073$ is a normalization constant equal to the maximum value of the function $\sin(\theta) e^{-|\theta|/\theta_{al}^0}$ for the upper cutoff of angle $\theta_{al}^0 = 0.2$ (11.5°), which represents that the magnitude of the aligning force and torque is reduced by a factor less than unity for angles larger than θ_{al}^0 [Ojic et al. 2011]. A maximum aligning force of 30 pN was implemented as for the upper limit in the contractile force.

A torque was introduced to rotate neighboring minifilaments along a common direction. The torque on minifilament i due to minifilament j is:

$$\tau_{rot}^{ij} = \tau_0 \left[\frac{\sin(\theta_1)}{C_{max}} e^{-\frac{|\theta_1|}{\theta_{al}^0}} \right] - \frac{\tau_0}{C_{max}} \left[\frac{\sin(\theta_2)}{C_{max}} e^{-\frac{|\theta_2|}{\theta_{al}^0}} \right] \quad (s < L_{max}) \quad (5)$$

where τ_0 is a constant representing the amplitude of the torque. Eq. (5) satisfies torque balance, $\tau_{rot}^{ij} = \tau_{rot}^{ji}$.

The alignment force and torque in Eqs. (4) and (5) use an exponential cutoff at approximately the same angle as the contractile force in Eq. (3). The sine terms represent a restoring, spring-like force/torque as function of angle that behaves linearly at small angles.

3. Short Range Repulsion (Fig. 6C) prevents minifilaments from fully overlapping with one another:

$$F_{repel}^{ij} = k_r (L_{min} - s) \quad (s < L_{min}) \quad (6)$$

We used $k_r = 50 \text{ pN}/\mu\text{m}$, which is large enough to counteract contractile forces. These forces are oriented along the line joining the centers of the minifilaments.

4. Stress-dependence minifilament dissociation (Fig. 6D). To simulate the dissociation of foci, we assumed that the lifetime of each minifilament is reduced by contractile forces:

$$k_d = k_{d0} \exp(-|F_c^i|/F_d^0) \quad (7)$$

where k_{d0} is the dissociation rate of non-interacting minifilaments, $|F_c^i|$ is the magnitude of the total contractile force experience by the minifilament, and $F_d^0 = 15 \text{ pN}$ defines a force scale over above which stress-stabilization occurs. When a minifilament dissociates from its current position, we place it at another random position and random orientation in the simulated cortical region thus keeping the total concentration constant. A value $k_{d0} = 0.001 \text{ s}^{-1}$ gave agreement with experimental results, see below.

At each time step ($dt = 0.01$ s) of our Monte Carlo simulation we calculate the total force $\mathbf{F}_{tot}^i$ on minifilament i , and update the positions $\mathbf{x}_i$ according to $\mathbf{x}_i(t+dt) = \mathbf{x}_i(t) + \left(\mathbf{F}_{tot}^i / \zeta\right) dt$. The value of the drag coefficient of the cortex is $\zeta = 400$ pN s/ μm that gives velocities that match those in experiments. The orientation ϕ_i of each minifilament is updated according to $\phi_i(t+dt) = \phi_i(t) + \left(\tau_{rot}^i / \zeta_{rot}\right) dt$, where τ_{rot}^i is the total torque experienced by the minifilaments and ζ_{rot} is the rotational drag coefficient. The independent parameter describing the rate of orientation change is τ_0 / ζ_{rot} . The parameters used in the simulations are summarized in Table I.

Simulation Results

To check if the model of the previous section reproduces basic features of the dynamics in the medial cortex, we run simulations in an $8 \times 8 \mu\text{m}^2$ region containing two focal adhesions (gray oval spots in Fig. 6E) at the two corners. The simulated density of focal adhesions is roughly the density in Type I and II control cells, respectively. The focal adhesions were each simulated as 9 static minifilaments oriented along the same permanent direction, representing the myosin minifilaments that would form at a fiber around the adhesion. In experiments, focal adhesions are dynamic and their growth depends on tension [Balaban et al. 2001; Choquet et al. 1997; Shutova et al. 2012; Stricker et al. 2011]. Here we are interested in myosin dynamics and for simplicity assume the adhesions are fixed. In the simulations the interaction between cortical minifilaments and those in focal adhesions is the same as the interaction between minifilaments, except that that the latter do not move during each simulating step.

To calculate the number of minifilaments to include, we measured the intensity of MRLC-GFP foci in experimental images and the mean intensity at medial regions of the cell cortex to estimate $N = 40 \pm 20$ (Mean $\pm$ StDev) in an $8 \times 8 \mu\text{m}^2$ region for control Type II cells. Fig. 6E shows a simulation starting with $N = 42$ randomly placed minifilaments. At the beginning of the simulation the minifilaments locally align and contract and build a tension-stabilized fiber at ~ 3000 s in between the focal adhesions, similar to what was observed in control cells with actomyosin bundles (Fig. 2A). This bundle is stable for ~ 1000 s when it breaks and starts to contract and disassembles in ~ 3000 s.

Type II cells have slightly less MRLC-GFP intensity and higher concentration of focal adhesions in the medial regions (Fig. S1). If we just decrease the number of myosin minifilaments in the simulations to $N=37$ (see Fig. 6E) we no longer obtain stable fibers in between the focal adhesions placed at the two corners. Thus a stable fiber network would require a higher density of focal adhesions, as observed in Type II cells. The third simulation in Fig. 6E shows that the model reproduces a transient network structure observed in Type III cells that have very few medial focal adhesions.

The probability of fiber formation across focal adhesions in simulations depends on the concentration of minifilaments, increasing continuously above a threshold value (Fig. 6F). This threshold behavior is similar to the threshold myosin concentration for formation of actomyosin fibers in vitro [Thoresen et al. 2011], the threshold myosin IIA concentration

required for bridges between cell adhesions [Rossier et al. 2010], and the cross-link threshold behavior for mechanically stable networks [Das et al. 2012]. The lifetime of the resulting fibers goes through a maximum (Fig. 6G) since in our model excessive number of minifilaments leads to transient short fibers along random directions that disrupt the main fiber linking the focal adhesions.

The simulations of Fig. 6 reproduce the correlation pattern quantified by STICS analysis in experiments. We checked this by performing radially-averaged STICS analysis on simulated images in which the shape of the minifilaments was a rectangle with dimensions $0.8 \times 0.2 \mu\text{m}$. Panels A, B and C in Fig. 7 and Movies 6, 7 and 8 show simulations and STICS analysis of Type I, II, and III cells (defined as in Fig. 6E). Similar to the analysis of the experiments in Fig. 3, we selected regions of size $5.6 \times 5.6 \mu\text{m}^2$ that excluded permanent fibers (see boxes in Fig. 7). We found that the STICS curves in the simulations of Fig. 7 have similar shape to those of Fig. 3F. The average decay time of $r(\rho, 0)$ is of order 100 s in all three cases, similar to Fig. 3F. Both contractile motions and minifilaments turnover contribute to establishing this relaxation time.

We checked if our proposed mechanism is consistent with the behavior of cells in blebbistatin. In Fig. 8 we simulated the effect of blebbistatin as a reduction in the contractile and aligning forces. The number of minifilaments included in these simulations was $N = 40$, similar to Fig. 7, since blebbistatin does not significantly change the MRLC-GFP intensity in regions of the medial cell cortex that do not contain permanent fibers (Fig. S1). By reducing the contractile and aligning force by a factor of ten (reducing k_c to $5.0 \text{ pN}/\mu\text{m}$ and F_{al} to 0.6 pN , approximately equal to the percent reduction of ATPase activity in $50 \mu\text{M}$ blebbistatin [Straight et al. 2003]) we find that minifilaments cannot assemble into long fibers. This happens with or without including focal adhesions in the simulations (Fig. 8A, C). Short fibers were still observed in the middle of simulated cortex; however, these fibers had a short lifetime and disassembled before they could elongate to bridge focal adhesions, maintain tension and stabilize. The reduction of the magnitude of contractile and aligning forces results in a reduction of the motion of the minifilaments. The decay time of the STICS $r(\rho, 0)$ in Fig. 8B, D is now 210-230 s, which is about twice longer compared to Fig. 8 because it is mostly determined by the turnover kinetics that occurs over a time $k_{d0}^{-1} = 10^3 \text{ s}$ rather than contraction and alignment. This increase in the decay time is in agreement with the results of Fig. 5.

In the simulations of the effect of blebbistatin in Fig. 8 we kept the parameters of minifilament turnover kinetics k_{d0} and F_d^0 in Eq. (7) unchanged compared to Fig. 7. While minifilament turnover still occurs in the presence of blebbistatin (Fig. 5B), it is difficult to accurately estimate the influence of blebbistatin on the minifilament turnover kinetics as a function of contractile force. For simplicity, we kept the turnover kinetics unchanged to show that the simulated reduction in contraction in Fig. 8 is enough to reproduce the experimental trend in the presence of blebbistatin. We also note that the FWHM of $r(\rho, 0)$ in Figs. 7 and 8 is somewhat smaller than in the experiments of Fig. 3 and 5 because the simulated minifilaments were drawn as $0.8 \times 0.2 \mu\text{m}$ rectangles, which did not include the effect of the microscope's point spread function broadening.

The mechanism described by the model can be used to make predictions about fiber growth when the location of focal adhesion formation is restricted (as for example on substrates with patterned adhesive regions). Movie 9 shows the results of a simulation with focal adhesions placed at the boundary of $20 \times 16 \mu\text{m}$ box with 252 minifilaments (a concentration $\sim 20\%$ larger than in Fig. 7A). The Movie shows fibers forming along the box boundary over the duration of the simulation (10^4 s) and a meshwork with decreasing number of minifilaments in the middle. The forces exerted on the adhesions increase with time, reaching a plateau after ~ 4000 s.

Discussion

The experimental and computational studies presented in this work support a picture of the medial actomyosin cortex existing in a dynamic state of assembly, reorganization and disassembly [Luo et al. 2013b; Rossier et al. 2010]. This state is on the verge of formation of long fibers and networks of fibers anchored at focal adhesions. This marginal behavior of the system leads to variability and history-dependence in cortical network structures in a population of cells. Here we divided cells into Type I, II and III, according to the length of the stress-fiber bundles relative to the length of the entire cell. Each type responds differently to external perturbations. When presented with a perturbation, such as a reduction in motor activity or a change in the extracellular environment, the medial cortex responds by reorganizing to a different network structure. To perturb the cortex here we used blebbistatin but similar concepts should apply to changes in signaling pathways that feed into cortical actomyosin dynamics or changes in the external mechanical environment that perturb the cell's ability to form anchors at focal adhesions.

The process of myosin foci appearance and disappearance likely reflects nucleation and growth of myosin minifilaments as well as myosin binding to actin filaments and other proteins near the cell membrane [Luo et al. 2012; Ren et al. 2009]. Myosin minifilaments can polymerize and bind to the cortex over 200 s and dissociate over a similar time [Luo et al. 2013a; Luo et al. 2012]. These times are similar to the lifetime of myosin foci used in the model. Here we assumed stress mainly influences the minifilament disassembly rate but the assembly rate can also be influenced by force [Luo et al. 2012; Verkhovsky et al. 1995]. The time lapse movies of MRLC-GFP show foci turnover in blebbistatin-treated cells (Fig. 5), however it is difficult to measure a difference with respect to control cells due to the difficulty in tracking individual foci as they contract and coalesce with one another.

The process of foci movement likely occurs by myosin pulling on actin filaments located at the cell cortex, leading to bundles with myosin minifilaments and actin cross-linking proteins. When coupled to adhesion formation, long fibers can develop along the cortex by maintaining tension through anchors at focal adhesions.

A limitation of our use of the radially-averaged STICS method is that we lose some information about local alignment dynamics. While the STICS analysis only highlights part of the dynamics, when combined with other methods of analysis (such as kymographs and montages, measurements of number of foci), it helps to better describe the contractile process. A challenge for future work is to develop and apply a correlation-based method

able to extract local alignment dynamics. Future FRAP experiments could also be helpful in separating turnover from contraction.

The effects of blebbistatin and of blebbistatin washout in the organization of actomyosin contractile structures has been studied extensively in a prior work [Shutova et al. 2012]. Both this prior study and our work show the disruption and re-formation of fibers and focal adhesions by blebbistatin. Here we focused on the medial cortex as opposed to adhesion and stress fiber formation from the lamellipodium and lamellar regions. In our system, the MRLC-GFP intensity in the medial cell cortex decreased for Type I cells in blebbistatin (Fig. S1D). This is consistent with the reported decrease in the fraction of polymerized myosin minifilaments in blebbistatin [Shutova et al. 2012], however we cannot distinguish between polymerized and unpolymerized myosin in our experiments.

Future experimental and modeling work could explore in more detail how signaling pathways in the cells contribute to the assembly and disassembly of myosin filaments and fiber stabilization [Besser and Schwarz 2007]. Phosphorylation regulates myosin minifilament assembly [Luo et al. 2012; Watanabe et al. 2007]. When fibroblasts and endothelial cells were treated with Y-27632, which inhibits myosin light chain phosphorylation by decreasing ROCK activity, the medial stress fibers were disrupted and focal adhesions disassembled, while most of the peripheral stress fibers were unaffected [Katoh et al. 2001]. When cells were treated with ML-7, an MLCK inhibitor, the peripheral stress fibers were observed to weaken but the medial stress fibers remained stable [Katoh et al. 2001; Watanabe et al. 2007]. Further investigation of time lapse MRLC-GFP images of cells with ROCK or MLCK inhibitors is required to better understand their role in cortical network reorganization dynamics.

Previous studies using lasers to cut stress fibers showed that the broken fiber ends recoil over several μm over 10 s [Kumar et al. 2006; Tanner et al. 2010], a process that has been modeled as sarcomeric contraction acting against viscous and elastic forces [Besser et al. 2011; Stachowiak and O'Shaughnessy 2009; Yoshinaga and Marcq 2012]. In our MRLC-GFP movies we also observe medial fibers breaking (Fig. S8), however this results in slower retraction and simultaneous disassembly over ~ 600 s (see Movie 10, event at ~ 55 min). MRLC-GFP foci exhibit stochastic redistribution around the shrinking fiber. These observations indicate that the transient fibers we observe in the medial cortex have different composition to mature stress fibers and/or are coupled in different ways to the surrounding cytoskeleton.

Our MRLC-GFP images of medial fibers and networks in HeLa cells were taken by focusing at the adhered part of the cell adjacent to the slides below the cell nucleus. 3D images of cells expressing MRLC-GFP and stained with vinculin antibody show networks and fibers anchored to the slide through focal adhesions, with very few connections to the cell nucleus. Connections of actin fibers to the nucleus however may be more pronounced in other cells [Chancellor et al. 2010]. Since the nucleus can provide anchor points [Tapley and Starr 2013] similarly to focal adhesions, the fibers connected to the nucleus may be stabilized by mechanisms similar to the ones in our model (O. Wiggan, private communication).

The model of fiber formation through coalescence of dynamic myosin foci of this paper describes similar dynamics to prior computational and mathematical models of cortical clump formation in LatA-treated cells [Luo et al. 2013b]. Our model further shows the importance of alignment and stabilization by stress for the formation of linear fibers. Since this process is mediated by actin filaments and actin-filament cross-linking, this may be why clumps, rather than fibers, prevail when actin filaments are depolymerized by LatA [Luo et al. 2013b]. Our results are consistent with studies in fission yeast cytokinesis that have shown that the interplay between contraction and cross-linking can result in actomyosin contractile rings, meshworks, or clumps depending on the concentrations of cross-linkers and actin dynamics [Laporte et al. 2012]. Here we did not explicitly include individual actin filaments but their role in bundle formation and aligning of actomyosin contractile systems has been demonstrated by earlier models [Borau et al. 2012; Cyron et al. 2013; Dasanayake and Carlsson 2013; Walcott and Sun 2010]. Future work could examine this process in more detail as well as the individual actin and myosin filament sliding within the fiber and formation of sarcomeric structures [Friedrich et al. 2012; Stachowiak et al. 2012; Yoshinaga et al. 2010], processes that our model did not address. Such studies, together with our work can provide a link to cell-level dynamics that have so far been studied with coarse-graining at a more macroscopic level compared to our work [Deguchi et al. 2011; Kaunas et al. 2011; Pirentis et al. 2011; Schwarz and Safran 2013; Stamenovic et al. 2009], mostly in the context of cell mechanosensing. Although our 2D model is simple, future extensions could be useful in modeling how elastic substrates of different stiffness develop cytoskeletal networks with different morphology and mechanical properties [Discher et al. 2005], and to investigate how the cytoskeletal network reorganizes in response to external perturbations such as cyclic stretching [Hayakawa et al. 2001] or shear flow [Sakamoto et al. 2010].

Materials and Methods

1. HeLa cell culture and treatment

HeLa cells stably expressing GFP-tagged myosin regulatory light chain (GFP-MRLC) were obtained from Dr. K. Kamijo [Kamijo et al. 2006]. The culture medium was prepared with the following ingredients: 500 mL Dulbecco's Modified Eagle Medium DMEM with high glucose (4.5%) (Invitrogen #11960-044); 6 mL Glutamine (200 mM) (Invitrogen #25030-081); 5 mL sodium bicarbonate (7.5%) (Sigma #S8761); 50 mL fetal bovine serum (10%, heat activated in 56°C for 30 minutes) (Invitrogen #10082-147); 5 mL penicillin-streptomycin antibiotic solution (10,000 U/mL) (Invitrogen #15140-122).

Live HeLa cells were cultured in a flask in a NuAire CO₂ incubator (5% CO₂ at 37°C) in 5 mL DMEM and split every 3 days with the addition of 40 µL antibiotic G418 sulfate (Invitrogen #10131-035). For live imaging, cells were dropped on 1 inch culture dishes with thin cover glass bottom and cultured for at least 48 hours before imaging or treatment with blebbistatin.

Blebbistatin (powder, Sigma #B0560) was dissolved in DMSO (Sigma #C6295) to make 100 mM solution and stored at -20°C. Blebbistatin solution (100 mM) was mixed with regular DMEM medium to make 100 µM or 50 µM blebbistatin medium. HeLa cells were incubated in blebbistatin medium for 60 minutes before staining for focal adhesions or actin

filaments. To wash out blebbistatin we used regular DMEM medium to wash the cell buffer 3 times. Washed-out cells were incubated in 5mL DMEM medium for 60 minutes before staining focal adhesions or actin filaments.

We performed control experiments to check that the results in Fig. 5A are not influenced by laser-induced inactivation of blebbistatin (the only case where we quantified live cells in blebbistatin). We did not see any noticeable change in myosin distribution after taking 10 images over 160 s. In each image of Fig. 5A we used 10% of maximum laser intensity and 2 $\mu\text{s}/\text{pixel}$ scanning speed (1 pixel = 62 nm). We observed a noticeable restoration of myosin fiber formation and contraction when using 30% of maximum intensity and 10 $\mu\text{s}/\text{pixel}$ scanning speed (Fig. S5). In Fig. S5 an additional initial scan with 30% of maximum intensity at 200 $\mu\text{s}/\text{pixel}$ at increased confocal aperture was performed. That the results of Fig. 5A are below the threshold of blebbistatin inactivation agrees with the previously reported threshold [Sakamoto et al. 2005]. These authors used similar imaging conditions to our study and found that 488 nm laser starts to inactivate blebbistatin at intensities above 0.1 $\text{mW}/\mu\text{m}^2$ when using 100 ms exposure time. The laser intensity used in Fig. 5 is also of order 0.1 $\text{mW}/\mu\text{m}^2$, however the exposure time per pixel is not larger than 2 ms when accounting for exposure due to scanning of nearby pixels.

2. Staining of actin filaments and focal adhesions

We cultured cells on coverglass for 2 days before staining them. The procedure of staining actin filaments and focal adhesions in HeLa cells is described in the following steps [Wylie and Chantler 2001]: (1) Cell fixation: cells were treated with 10% formalin solution (3.7% formaldehyde in PBS) for 15 minutes. (2) PBS saline solution was used to rinse 3 times, for 2 min each time. (3) Permeabilization: 0.1% Triton X-100 was used for 15 min. (4) Blocking: 1% BSA was used for 30 min. BSA was stored in a -20°C freezer. (5) Rhodamine phalloidin was used to label actin filaments and vinculin primary antibody to label focal adhesions. The dye and antibody were used at 1:100 concentration (500 μL on each coverglass). The antibody and dye solution was allowed to react for at least 1 hour at 37°C . For imaging at a different day the sample was covered in aluminum foil stored at 4°C . (6) PBS solution was used to rinse 3 times (2 minutes each time). (7) Nuclei were also stained with Hoechst dye (0.002 mg/ml in aqueous solution) for 2-5 min. (8) dH_2O was used to rinse off the unbound dyes 3 times (2 minutes each time). The sample was then washed and stored in PBS solution.

3. Microscopy and image processing

We used an Olympus FV1000 confocal microscope to take time-lapse images of HeLa cells. The lens was an Olympus UPLAN 100X oil immerse objective ($\text{NA}=1.3$). We focused on the adhered part of the cells above the glass slide. Multi-channel excitation allowed us to obtain images of MRLC-GFP, actin filaments, focal adhesions and nuclei of cells after staining them with different fluorescence dyes. We used the following settings consistently, to achieve the best S/N and minimum photo-bleaching: Scanning speed: 2 $\mu\text{s}/\text{pixel}$; Image resolution in x-y plane: 62nm/pixel; 10% of maximum power of excitation laser; HV=640V; offset = 10.

Photobleaching correction was performed as follows. Firstly, we subtracted the out-of-cell background separately at each frame. We then calculated total intensity of each slice vs. time. Next, we fit this curve with a single exponential decay $I(t) = I_0 \exp(-t / t_0)$ to obtain I_0 and t_0 . Finally, we multiplied each frame with $\exp(t / t_0)$.

Quantification of cell images. The cell boundary was manually selected in ImageJ. The MRLCGFP intensity within the selected region (ROI) was measured on the photobleach-corrected images. The cell spread area was measured by fitting an active contour to the cell boundary using JFilament2D [Smith et al. 2010]. To measure the number of focal adhesions in vinculin-stained cells we binarized the images, applied a band-pass filter and used the analyze particles function in ImageJ, see Fig. S2 and [Huang et al. 2010].

4. STICS analysis and correction for white noise background

We developed an ImageJ plugin to perform the STICS analysis in Eqs. (1) and (2) with the help of M.B. Smith. We applied STICS after correcting for photobleaching on regions of the medial cell cortex with small intensity fluctuations, as explained in the main text. The resulting STICS function has a sharp peak at $r(0,0)$ due to white noise [Wiseman and Petersen 1999]. We removed this peak by fitting the $r(\rho,0)$ curve with a Gaussian, using $\rho > 1.5$ pixels and used the curve to extrapolate to $r(0,0)$ [Wiseman and Petersen 1999].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Actomyosin fibers and networks in HeLa cells adhered to glass slides. (A) Fluorescence microscopy images (single confocal slice through the bottom part) of control cells showing myosin labeled with MRLC-GFP and actin filaments stained with rhodamine phalloidin. (B) Images of cells expressing MRLC-GFP and focal adhesions stained with vinculin antibody. All cells show peripheral stress fibers but structures in the medial cell part vary among cells on the same glass slide. We classify cells into three types ($n = 70$ cells for A and $n = 41$ in B). In Type I cells, medial myosin and actin form fibers of length comparable to the cell size; these fibers connect focal adhesions located at the boundary or in the middle cellular region. In Type II cells, medial myosin and actin form short fibers and networks anchored by focal adhesions located at the boundary and in the middle cellular region. In type III cells, there are no detectable medial fibers, networks or focal adhesions. (C) Comparison of ratio between average MRLC-GFP intensity in cell middle and whole cell within a single confocal slice through the bottom part of the cell ($n = 41$). Type I and Type II cells have larger a larger ratio compared to Type III. (D) Total number of focal adhesions for three cell types ($n = 41$). Type II cells have the most adhesions in the cell middle while Type III have

close to none. *: $p < 0.05$; **: $p < 0.01$ (since values in each bin come from the same sample after manual classification, the p-values here are provided as a guide). Bars: 10 μm .

Figure 2.

Time-lapse fluorescence microscopy images of HeLa cells expressing MRLC-GFP reveal a dynamic cortical network in which MRLC-GFP foci appear, disappear, clump, align, and contract. Single confocal slices through cell bottom. Montage panels to the right of A, B, C correspond to white box of left panel. (A) MRLC-GFP foci in a Type I cell assemble into linear structures within 7 min (Movie 1). Other linear structures are observed to disassemble in Movie 1. (B) MRLC-GFP foci appearance/disappearance and contraction in a Type II cell (Movie 2). In this cell the intensity of the medial MRLC-GFP network increases towards the periphery. MRLC-GFP foci in the medial region flow, on average, towards the cell periphery. (C) MRLC-GFP foci appearance/disappearance and contraction in Type III cells (Movie 3). The top right cell can also be classified as Type II. (D) 3D kymographs of

montages of panels A, B and C. Foci appearance and disappearance are evident as bright spots (red arrowheads). Contraction corresponds to diagonal features in the kymographs (green arrowheads). Total time is indicated in each panel. Bars: 5 μ m.

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Figure 3.

STICS analysis of MRLC-GFP dynamics in control cells at a single confocal slice at cell bottom. (A) Montage picture (right) of selected region in the middle of a Type II HeLa cell (left). Bars: 10 μm (left) and 5 μm (right). For analysis of steady state dynamics we selected regions of size 100x100 pixels (1 pixel = 0.6 μm) that lack stable fibers over 160 s and do not change in average intensity by more than 2%. (B) Snapshots of STICS function $r(\zeta, \eta, \tau)$ versus time τ show its approximate radial symmetry around the origin $\zeta = \eta = 0$ at the center of the image. (C) Percent change of intensity of selected region over time shows fluctuations within 0.05% of the average intensity. (D) Radially-averaged spatial-temporal image correlation function of selected region as function of radial distance ρ and delay time. (E) Single exponential fit of the correlation function of panel C at $\rho = 0$ gives a characteristic decay time $\tau = 81$ s. (F) Normalized decay curves (at $\rho = 0$) in different control cells. The

average decay time of individual exponential fits gives $\tau_{\text{control}} = 130 \pm 50$ s (Mean $\pm$ StDev, $n = 25$).

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Figure 4.

Images of HeLa cells expressing MRLC-GFP (green) stained with vinculin (red) after treatment with blebbistatin. (A) Cells treated with 100 μ M blebbistatin for 60 min. Red arrow indicates fibers in Type I cells that remain after treatment. (B) Cells treated with 100 μ M blebbistatin for 60 minutes followed by washout with regular DMEM medium. Images were taken 60 min after the washout. Bar: 10 μ m.

Figure 5.

STICS analysis of MRLC-GFP dynamics of cells treated with blebbistatin (A-D) or of cells recovering from blebbistatin (E-H), at a single confocal slice at cell bottom. (A) Cell after treatment with 50 μM blebbistatin for 60 minutes. Right: montage of selected region. Bars: 10 μm (left) and 5 μm (right). For analysis of steady state dynamics we selected regions of size 100×100 pixels (1 pixel = 0.6 μm) that lack stable fibers over 160 s and do not change in average intensity by more than 2%. (B) 3D kymograph of montage of panels A. Foci appearance and disappearance is observed but the near absence of diagonal features indicates less contraction compared to control cells. (C) Radially-averaged spatial-temporal image correlation function of selected region as function of radial distance ρ and delay time. (D) Normalized decay curves (at $\rho = 0$) in different blebbistatin-treated cells. Line with dots corresponds to the region in panel A and thick line is the average. The average decay time of

individual exponential fits gives $\tau_{bleb} = 240 \pm 90$ s (Mean $\pm$ StDev, $n = 24$). (E) Cell treated with 50 μ M blebbistatin for 60 minutes and imaged 60 min after washout with regular DMEM medium. Right: Montage of selected region. Bars: 10 μ m (left) and 5 μ m (right). (F) 3D kymograph of montage of panel E. Foci appearance and disappearance as well as diagonal features indicating contraction are observed. (G) Radially-averaged spatial-temporal image correlation function of selected region in panel E. (E) Normalized decay curves (at $\rho = 0$) in different cells recovering from blebbistatin. Line with dots corresponds to the region in panel E and thick line is the average. The average decay time of individual exponential fits gives $\tau_{washout} = 220 \pm 160$ s (Mean $\pm$ StDev, $n = 23$).

Figure 6.

Cartoon of coarse-grained model of myosin minifilament interaction and results of simulations of Type I, II and III cortices. (A) The contractile force $\mathbf{F}_c^{ij} = -\mathbf{F}_c^{ji}$ between two minifilaments i and j is determined by angles θ_1 , θ_2 , and d . And is applied when $L_{min} < d < L_{max}$. The contractile force increases with decreasing $|\theta_1|$, $|\theta_2|$ and increasing d . The contractile force is assumed to be mediated by actin filaments that are not explicitly included in the model. (B) Minifilaments experience torques $\tau^{ij} = -\tau^{ji}$ that rotate them to point toward each other when $d < L_{max}$. Aligning forces $\mathbf{F}_{al}^{ij} = -\mathbf{F}_{al}^{ji}$ act on the center of each minifilament, perpendicularly to the line joining their centers. These torques and forces are also assumed to be mediated by actin filaments as in panel A. (C) Excluded-volume short-range repelling forces $\mathbf{F}_{repel}^{ij} = -\mathbf{F}_{repel}^{ji}$ are applied when $d < L_{min}$. (D) The dissociation rate for each minifilament decreases exponentially with increasing total contractile force acted on the minifilament. This results in stabilization of linear structures that bear tension. Dissociated minifilaments are placed at a random position on the plane simulating the cell cortex to maintain constant overall minifilament concentration. (E) Simulations of Type I, II and III cells. Top snapshots: Fibers form in between focal adhesions (simulated as stationary minifilaments indicated by arrows) when using a contractile force spring constant $k_c = 50$ pN/ μ m and aligning force magnitude $F_{al} = 6$ pN in a $8 \times 8 \mu\text{m}^2$ region. The bundle is stable

for ~2000 seconds, similar to structures observed in Type I cells. Middle snapshots: with k_c and F_{al} decreased by 10% bundles are not stable in between the adhesions; a transient network forms in between them, similar to Type II cells. Bottom snapshots: in simulations with the same k_c and F_{al} as in the middle, but without adhesions, a transient network forms as in Type III cells. (F) Probability to form fiber in between focal adhesions as function of number of minifilaments in simulations of panel E ($n = 30$ simulations for each case, lasting for 10^4 s). (G) Average lifetime of fibers as function of number of minifilaments for the same simulations as panel F. Error bar: standard deviation. The lifetime goes through a maximum since excessive number of minifilaments leads to many transient short fibers along random directions that disrupt the main fiber linking the focal adhesions.

Figure 7.

Application of STICS to simulated images reproduces correlations seen in control cells. Analysis is performed as in Fig. 3. The decay time obtained in all cases is close to the decay time from experimental data of Type I and Type II cells in Fig. 3. (A) Montage of selected region from image of size $8 \times 8 \mu\text{m}$ that contains two simulated focal adhesions and parameters corresponding to Type I cells. Bottom: radially-averaged spatial-temporal image correlation function of selected region as function of radial distance ρ and delay time and single exponential fit of the correlation function at $\rho = 0$ gives a characteristic decay time τ

= 105 s ($n = 10$). (B) As panel A, for parameters corresponding to Type II cells and two focal adhesions (C) As panel B, without focal adhesions, corresponding to Type III cells.

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Figure 8.

2D simulation of myosin network in Type II and Type III cells treated with blebbistatin (A, B: with focal adhesions; C,D: without focal adhesions). The effect of blebbistatin was simulated as a decrease of the contractile and aligning forces by 90% ($k_c = 5.0$ pN/ μm , $F_{al} = 0.6$ pN) compared to in Fig. 6. No fibers formed in either (A) or (C) and minifilaments are mostly stationary. (B,D) STICS analysis on the simulated cells treated with blebbistatin demonstrates that decay time increases ($\tau = 210$ s with focal adhesions and $\tau = 230$ s without) consistent with the experimental data in Fig. 5.

Table I

Parameter values used in simulations. For justification see main text unless otherwise indicated.

Parameter	Value
k_c	50 pN/ μ m
F_{al}	6 pN ^a
k_r	50 pN/ μ m
$L_{\max}$	1.6 μ m
$L_{\min}$	0.8 μ m
θ_c^0	0.3 rad
θ_{al}^0	0.2 rad
k_{a0}	0.001 s ⁻¹
F_d^0	15 pN
ζ	400 pN·s/ μ m ^b
ζ_{rot}/τ_0	400 s/rad ^c
dt	0.01 s

^aEstimated to be comparable to contraction force.

^bValue that gives minifilament speeds similar to those in experiments

^cEstimated to give a minifilament rotational speed similar to translational speed.