Interplay of RhoA and Motility in the Programmed Spreading of Daughter Cells Postmitosis

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ABSTRACT Upon cortical retraction in mitosis, mammalian cells have a dramatically decreased physical association with their environment. Hence, mechanisms that prevent mitotic detachment and ensure appropriate positioning of the resulting daughter cells are critical for effective tissue morphogenesis and repair, and are the subject of this study. We find that, unlike low-motility cells, highly motile cells spread isotropically upon division and do not typically reoccupy their mother-cell footprint, and often even disseminate their mitotic cells. To elucidate these different motility-based phenotypes, we investigated their partial recapitulation and rescue using defined molecular perturbations. We show that activated RhoA is localized at the mitotic cell cortex, and Rho-associated kinase inhibition increases the degree of reoccupation of the mother-cell outline in highly motile cells. Conversely, we show that induction of motility in low-motility cells by RasV12 overexpression results in increased isotropic daughter-cell spreading. We thus propose that a balance between cortical retraction forces, which depend in part on RhoA activation, and substrate adhesion forces, which diminish with increasing motility rates, governs the integrity of mitotic actin retraction fibers and influences subsequent daughter-cell spreading. This balance of forces during mitosis has implications for cancer metastasis.

INTRODUCTION

A cell's niche plays a critical role in maintaining its phenotype (1). In particular, control of cell division and appropriate positioning of daughter cells postmitosis is crucial for embryogenesis and for regulated tissue growth, repair, and homeostasis (2). It is important to decipher the precise role of cell- extracellular matrix (ECM) interactions during this process because most mammalian cells completely round up during division. Cells may possess mechanisms that prevent this transient loss of shape anisotropy from potentially hindering the correct positioning and efficient spreading of the resulting daughter cells. Indeed, early studies showed that in certain cells, such as PtK2, the daughter cells spread within the interphase footprint of the mother cell (3–5). These studies also identified a critical role for actin retraction fibers in this process. Specifically, they showed that these fibers appear to guide daughter-cell spreading on unpatterned surfaces postmitosis. Furthermore, recent studies using patterned surfaces showed that when spatial polarization was imposed, the orientation of the spindle during division became aligned with the major axis of the mother cell (6,7). This role for extrinsic cues in spindle positioning has also been seen in vivo (8,9). Mechanistically, although RhoA activity has been shown to be partially involved in mitotic cell retraction and cortical stiffening (10), a major contribution of the ezrin-radixin-moesin proteins to the regulation of mechanical changes in the cell cortex during mitosis has also recently emerged (11,12). These results suggest that cortical mechanical heterogeneity during mitosis, which is a consequence in part of the architecture of the actin cytoskeleton and associated cell-ECM interactions of the interphase mother cell, helps guide the spindle orientation (13) and hence the positioning of daughter cells postmitosis.

However, there are several aspects of the cell division process that are not completely understood. In particular, different cell types show different extents and durations of their association with the ECM. This may be due to differences in their intrinsic motility (which affects overall substrate adhesion (14)), or to distinct niche properties such as those observed when cell-cell contacts are more prevalent. It is unknown whether all such cell types process ECM cues in a similar manner during division. Therefore, in this study we explored the nature and role of cell-ECM interactions during cell division, and especially their influence on daughter-cell spreading, using cells with different motility rates as a model system. We investigated cell cytoskeleton and DNA dynamics during mitosis and daughter-cell spreading patterns postmitosis using lines stably expressing green fluorescent protein (GFP)-tagged actin, tubulin, or histone H2B proteins, and also tracked activated-RhoA dynamics using lines stably expressing a genetic RhoA fluorescence resonance energy transfer (FRET) sensor (15). We show that upon division, high- and low-motility cells have dramatically different daughter-cell spreading phenotypes. To elucidate these differences, we partially recapitulated and rescued them using defined molecular perturbations. From these results, we conclude that daughter-cell spreading depends on the mitotic cell-substrate attachment

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footprint, which in turn is governed by a balance of substrate adhesion and cortical retraction forces during cell division. Furthermore, induced gain or loss of motility can reciprocally modulate the above two forces, and this balance of forces also has important implications for cell dissemination (and hence metastasis) during mitosis.

MATERIALS AND METHODS

ECM micropatterning

Micropatterns for the stamp masters were created using AutoCAD with feature sizes between 5 and 50 μm. The micropatterns were chrome-printed onto soda lime glass. These masks were used to create the masters with standard photolithography techniques using SU-8 2005 (MicroChem, Newton, MA) as the photo resist and 4-inch Si wafers as the substrate. For improved adhesion, Piranha cleaning and use of OmniCoat (MicroChem) were found to be critical. Polydimethylsiloxane (PDMS) stamps (Sylgard 184 silicone elastomer kit; Dow Corning, Midland, MI) were made using the standard 10:1 ratio of elastomer to curing agent. After the PDMS was cured at ~70°C for at least 6 h, it was carefully peeled off and the desired patterns were cut out to obtain the stamps. The stamps and stamp master were functional for several stamping iterations. The procedure for stamping was as follows: Glass coverslips were first placed into a 2–5% solution of 3-mercaptopropyl-trimethoxysilane in ethanol or acetone. The coverslips were immersed in this solution for 20 min before being removed and dried thoroughly with N_2. The stamps were then inked with a 50 μg mL^{-1} solution of fibronectin for 10 min. After this inking period, the stamps were dried thoroughly with N_2 before being inverted onto the coverslips. Stamps were left for ~1 min before being removed. After stamping, the coverslips were placed into a solution of 100 μg mL^{-1} mPEG-Malemide (Sigma, St. Louis, MO) or 0.1% pluronic for ~15 min to 2 h before the cells were plated.

Cell culture

The cells used in this study were either human ovarian surface epithelial (OSE) cells or HeLa cells. The former is a highly motile cell type, whereas HeLa cells have relatively low motility. Cells were grown in Dulbecco's modified Eagle's medium + 10% fetal bovine serum under standard conditions. Plasmids expressing GFP-Actin (a gift from Dr. Edidin, Department of Biology, Johns Hopkins University), Histone2B-GFP (Addgene 11680, Addgene, Cambridge, MA), GFP-tubulin, hRasV12, and wt-RhoA-FRET-biosensor (Addgene 12150) were used for real-time visualization of the indicated proteins. To ensure reproducibility and avoid issues related to cell death associated with transient expression of some of the above genes, stable lines expressing these vectors were made in all cases. Typically, cells were analyzed 3–4 weeks after initial transfection. Y27632 (Calbiochem, EMD Biosciences, San Diego, CA) treatment of cells (10 μM) was initiated at the start of time-lapse experiments.

Imaging

We used live-cell imaging to track the large-scale morphological features of dividing cells with a specific focus on actin, mitotic spindle, metaphase DNA, and activated RhoA dynamics. Images were recorded using a Nikon TE-2000E with perfect focus. Time-lapse, multipoint, multiphase imaging was conducted for 15 h periods, and images were collected every 5–15 min. Longer time intervals (e.g., 15 min) were preferable for some stable lines, such as GFP-tubulin, because this minimized photobleaching and fluorescence-related toxicity. The live-cell settings were 37°C, 5% CO_2, and 75% relative humidity. Images were acquired using NIS-Elements Advanced Research Edition 2.30 Build 387. Image analysis was performed using both NIS-Elements and ImageJ. The statistical significance was computed using the Student's *t*-test for a two-tailed distribution and two-sample unequal variance. For morphological analysis, the circularity ratio was measured as $4\pi A/p^2$, where A is the cell footprint (projected area) and p is the perimeter. The normalized cell perimeter is the perimeter at time t divided by the maximum perimeter. Identical results were obtained using the software or from analysis of the number of pixels.

RESULTS

Spreading of daughter cells postmitosis in low-motility versus high-motility cells

For identical adhesive ligand densities on a substrate, cells induced to motility have a relatively weaker adhesion to their substrate (14). Thus, to gain insight into the role of cell-ECM interactions, we evaluated the global morphological changes in cell shape during division of highly motile versus low-motility cells. For the former we chose human OSE cells, and for the latter we chose HeLa cells (motility rates: OSE $= 4.1 \pm 0.3$ nm s^{-1}; HeLa $= 1.9 \pm 0.1$ nm s^{-1}; $p < 0.005$; see Fig. S1 *A* in the Supporting Material). Lines for each cell type stably expressing individually either GFP-actin or GFP-tubulin were established to visualize cytoskeletal dynamics, and in addition lines stably expressing GFP-H2B as a surrogate marker for visualizing DNA dynamics were also established.

For low-motility cells we found, consistent with earlier reports (3–5), that upon division the daughter cells spread within the footprint of the mother HeLa cell (Fig. 1 *a*, Movie S1). This was observed for both single cells and cells growing in clusters. Upon closer examination, we found that the dynamics of division and daughter-cell spreading underwent characteristic changes quantified by a time-dependent circularity ratio (Fig. 1 *b*). Upon initiation of division, the cellular circularity ratio increased due to the rounding of the mother cell (phase I), followed by a plateau phase in which the cell and the underlying mitotic machinery were repositioned (phase II). This repositioning was associated with the realignment of the mitotic spindle/metaphase DNA. Daughter-cell spreading after division was characterized by two distinct steps, which ultimately led to a final circularity ratio similar to that of the mother cell. The first step (phase III) consisted of rapid daughter-cell spreading along the retraction fibers that appear to serve as guides. In the second step (phase IV), the daughter cells spread out more slowly, usually in the direction perpendicular to these radial fibers, to fill the mother cell outline, likely also using other remnant ECM cues left behind during initial rounding. The excellent agreement between the initial and final circularity ratios highlights the fact that the daughter cells spread to occupy the original footprint of the mother cell. The latter phase (phase IV) of daughter-cell spreading is not unexpected since migrating cells have been known to leave behind focal adhesion components (16) that serve as nucleation points for

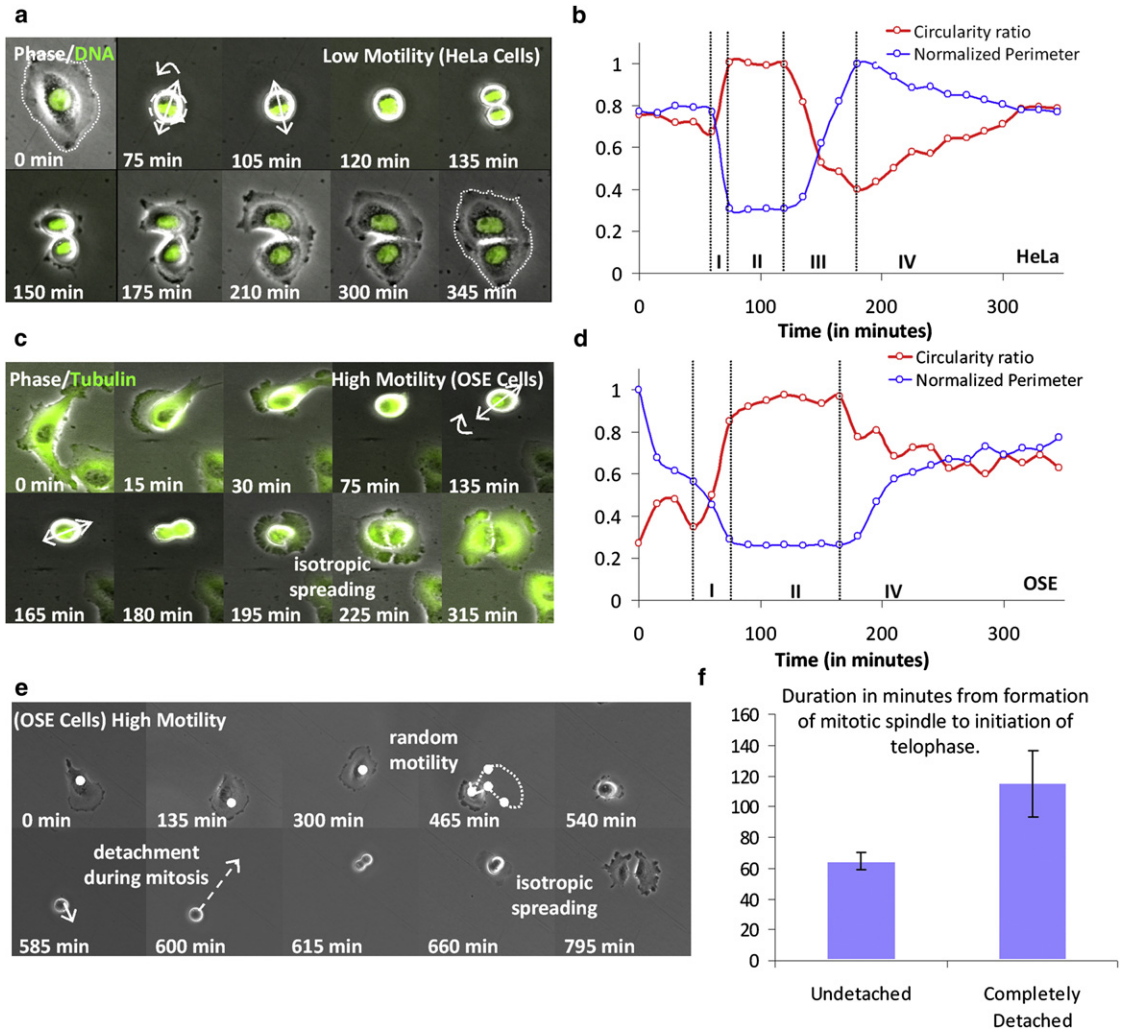

FIGURE 1 (*a*) Low motility cells (such as HeLa cells) typically divide broadly within the outline of the interphase shape of the mother cell. (*b*) A closer examination reveals that this process is characterized by four morphologically distinct phases (evaluated using either normalized perimeter or circularity ratio). Upon initiation of division, there is a rise in circularity ratio due to cell rounding (phase I), but the cells retain substrate contact through visible actin retraction fibers. This is followed by a plateau phase (phase II), where the mitotic machinery, specifically the mitotic spindle and metaphase DNA, repositions itself (see *arrows*). Finally, upon division there is a two-step return to a final circularity ratio similar to that of the mother cell. In the first step (phase III), the daughter cells spread rapidly along the actin retraction fibers that serve as guides, resulting in a sharp drop in the circularity ratio. Finally, in the second step (phase IV), the daughter cells spread out more slowly to fill the remnant mother cell footprint. The circularity ratio here is defined as $4\pi A/p^2$, where A is the cell footprint (projected area) and p is the cell perimeter. The normalized cell perimeter is the perimeter at time t divided by the maximum perimeter. (*c*) For high motility cells (such as OSEs), the daughter cells spread isotropically upon division, and thus do not typically reoccupy the mother cell footprint. (*d*) Consistent with this, the circularity ratio for OSEs also does not show a rapid initial spreading of daughter cells (phase III). (*e*) Furthermore, unlike HeLa cells, for OSEs there is often also detachment of the mitotic cells from the substrate, and (*f*) such detached cells spend a markedly increased time in cell division (mean $\pm$ SE, $p = 0.049$).

preferential reformation of adhesions (see also Fig. S1). Together, these observations imply that local cortical connections and cues relayed in part through actin retraction fibers play an important role in guiding daughter-cell spreading postmitosis.

The above observations were, however, in stark contrast to our findings in OSE cells, a highly motile cell type (Fig. 1 *c*). In this case, the dividing daughter cells did not usually reoccupy the footprint of the mother cell, but instead spread isotropically. As a result of this isotropic spreading, these high-motility cells do not typically show a phase III of rapid spreading to anchor points of an interphase outline (Fig. 1 *d*). Furthermore, we also observed that OSE mitotic cells had an increased susceptibility to detach from the substrate (Fig. 1 *e*). These detached mitotic cells could subsequently complete cytokinesis only upon reattachment to the substrate at another neighboring random location, and consequently these cells also took significantly longer to complete cell division (Fig. 1 *f*). Together, these observations imply that highly motile cells, consistent with their more transient and weaker substrate association, process fewer ECM cues during mitosis as compared to low-motility cells.

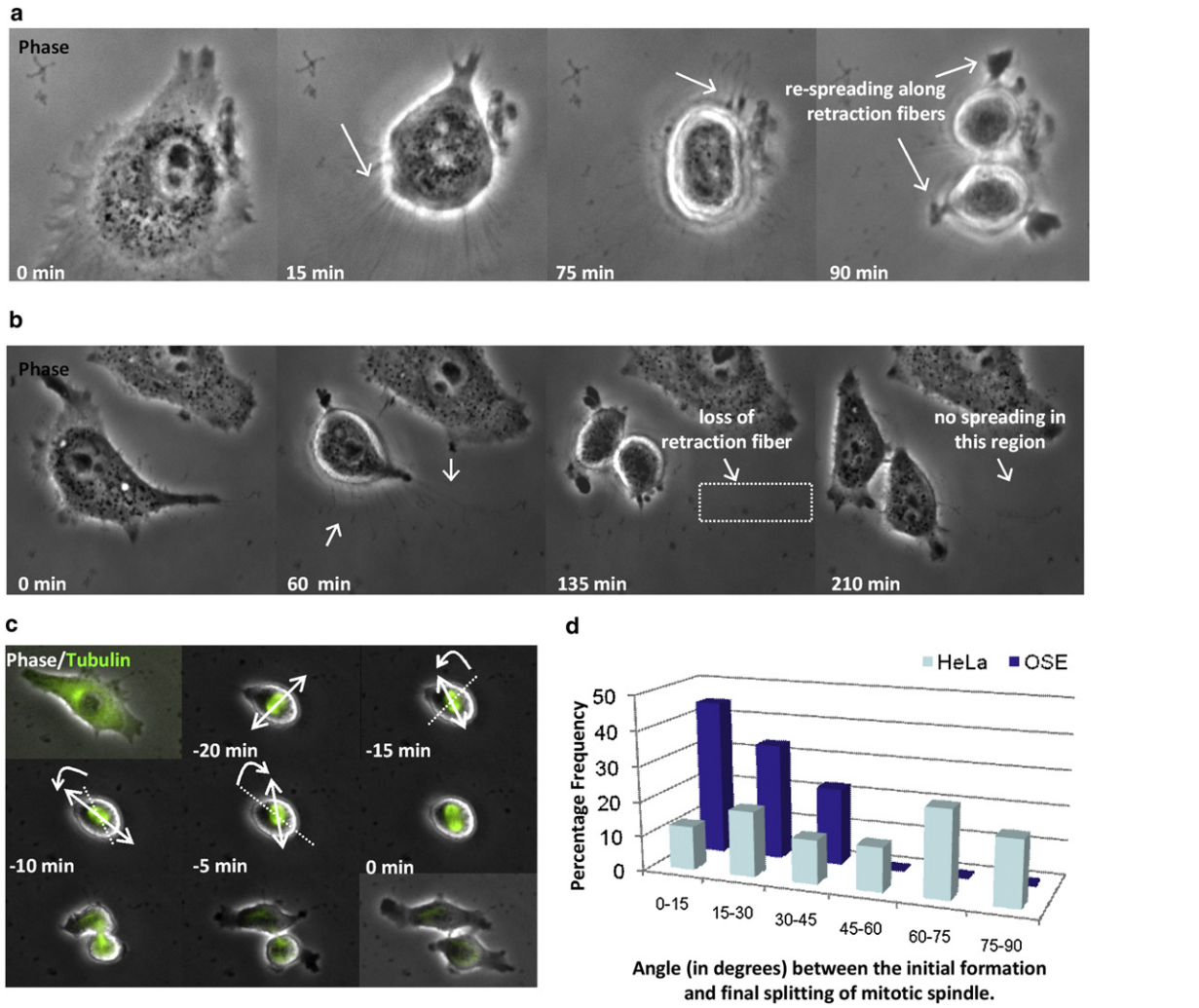

FIGURE 2 (*a*) Actin retraction fibers observed during cell rounding remain taut throughout the division process and appear to serve as guides during respreading of the daughter cells. In particular, there are distinct waves of actin polymerization and subsequent lamellopodia formation along these after cytokinesis (see *arrows*). (*b*) Consistent with Mitchison (4), their role is further supported by the observation that a spontaneous loss of a retraction fiber prevents daughter cell spreading into the associated region. (*c*) Furthermore, we also observe that cell-ECM interactions are also manifest in the dynamics of the underlying mitotic machinery. Specifically, tracking of the underlying spindle dynamics reveals that reorientation towards the final splitting axis is typically associated with both a net shift in overall alignment with respect to the initial formation position (a function of ECM cues (6)), and also the transition trajectory is accompanied with a characteristic oscillatory spindle motion with overshoots (a function of stochastic motor protein attachment (17)). (*d*) Specifically, we find that the angular spread between the initial orientation of the spindle versus the final splitting axis is greater for low motility cells (HeLa) versus high motility cells (OSEs), thus further implicating a differing role of ECM cues between the two cell types.

These different motility-based phenotypes prompted us to investigate more closely the role of substrate adhesion and ECM cues that cells use during the course of cell division. We also investigated the molecular machinery that might mediate such interactions, and sought to determine whether the different cell division phenotypes can be reconciled.

Role of substrate adhesion and retraction fibers in spindle dynamics and daughter-cell spreading

Because of the highly accurate reoccupation of the interphase mother-cell footprint on daughter-cell spreading of low-motility cells, we first used live-cell imaging to explore the cytoskeletal dynamics during mitosis and subsequent spreading of daughter cells in HeLa. Tracking of actin dynamics using a cell line stably expressing GFP-actin confirmed, consistent with earlier reports (3–5), that the retraction fibers are not merely passive actin-rich cell debris left behind during the rounding phase of the mother cell. First, the fibers formed a continuous phase with the cytoplasm of the mother cell, as cytoplasmic material was detectable along their entire length. Second, postmitosis there were distinct outward radial waves of actin polymerization and cytoskeletal proteins along the retraction fibers, guiding the spreading of the daughter cells to the original footprint of the mother cell (Fig. 2 *a*, Movie S2). Third,

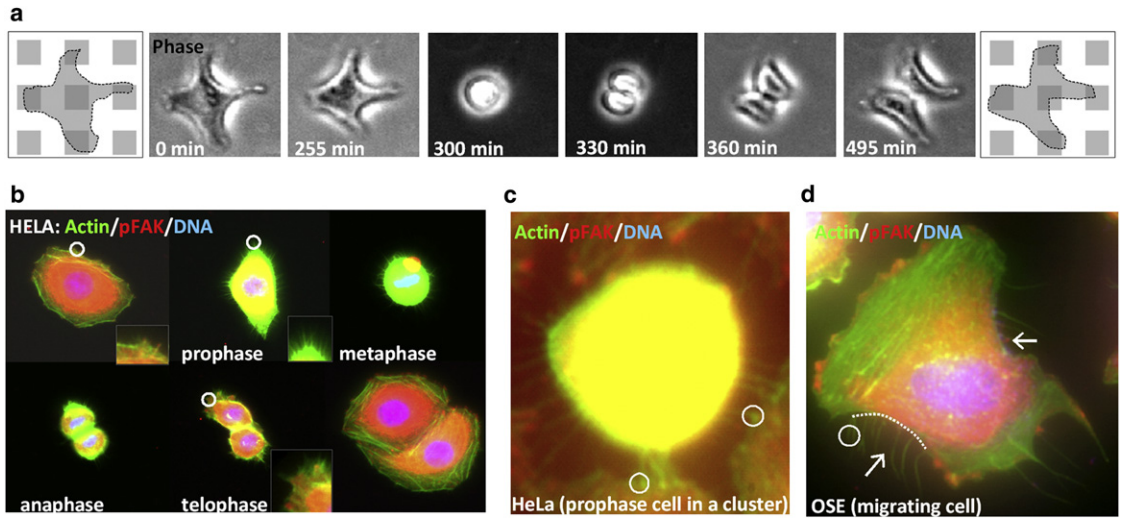

FIGURE 3 (*a*) For HeLa cells, if we fix the regions of adhesion of the mother cell to the substrate using micropatterned fibronectin islands of subcellular dimension (here 10 μm $\times$ 10 μm), we find that the daughter cells spread to precisely reoccupy these key points of attachment. (*b*) Correlated with this, phosphorylated (pT397) FAK (activated FAK) is rapidly lost from focal adhesions upon initiation of cell division, as evidenced by its absence at the ends of the actin fibers starting in early prophase, and is again recruited only during late telophase. (*c*) However, the ends of the actin retraction fibers continue to maintain a degree of substrate adhesion (particularly visible in cell clusters). (*d*) Analogously, in migrating cells, pFAK is lost from the retracting ends (now bearing remnant actin-rich fibers), but the advancing edges have strong FAK activity.

a spontaneous loss of any fiber prevented daughter-cell spreading into that region (Fig. 2 *b*, Fig. S2). Together, these results imply that the integrity of actin retraction fibers is critical for conserving the mother-cell outline in the spreading of HeLa daughter cells.

Although actin retraction fibers appear to guide the spreading of daughter cells, previous studies have suggested that their spatial organization and density also influence the alignment of the mitotic spindle and the axis of cell division (6,7). However, those studies relied on immunofluorescence microscopy of fixed cells, whereas in this work we used stable lines expressing H2B-GFP and GFP-tubulin to perform live-cell imaging of DNA and mitotic spindle dynamics (Fig. 2 *c*). We observed that the initial formation of the metaphase DNA plate and the associated mitotic spindle were often away from the final daughter-cell splitting axis, and consequently a net realignment of the spindle occurred during mitosis. We also observed that this realignment trajectory did not always asymptotically approach the final axis of splitting, but often exhibited an overshoot about local spindle positions, resulting in characteristic oscillations (Fig. 2 *c*, Movie S3). Although stochastic motor protein attachment and detachment can cause these latter spindle oscillations (17), a role for the ECM cues in guiding the spindle positioning (supporting the findings of Théry et al. (6)) is implied by the convergence of these oscillations with a net shift from their starting position. This realignment process, however, showed significant differences between the high- and low-motility cells. For HeLa cells, the initial formation of the spindle showed a large angular distribution about the final splitting axis (Movie S4). In contrast, for OSE cells, the angular distribution was much smaller (Fig. 2 *d*) and the spindles showed diminished realignment dynamics. These results further lead us to conclude that ECM cues influence spindle alignment, and that their influence appears to be greater in low-motility cells (HeLa) as compared to highly motile cells (OSE).

To further confirm the role of cell-substrate adhesion cues in the above process, we next investigated focal adhesion dynamics during division of HeLa cells. Using microcontact printing to pattern precise regions of adhesion at subcellular dimensions (here 10 μm $\times$ 10 μm) for the mother cell, we found that even though all apparent shape information was lost due to cell rounding during division, the daughter cells spread to reoccupy previous points of attachment of the mother cell (Fig. 3 *a*, Movie S5). Furthermore, phosphorylated (pT397) FAK (activated FAK (18); Fig. 3 *b*) was rapidly lost from focal adhesions upon initiation of cell division, as evidenced by its absence at the ends of the actin fibers starting in prophase (and upon initiation of cell retraction). However, the ends of the actin fibers appeared to continue to maintain adhesion to the substratum, and this anchoring was structurally pronounced in cell clusters (Fig. 3 *c*). Activated FAK was again recruited only during late telophase, and these sites proceeded to develop into mature focal adhesions (Fig. 3 *b*). A similar conclusion could be drawn for migrating cells (Fig. 3 *d*), where pFAK was lost from the retracting ends, which bore remnant actin-rich fibers and often whole remnant focal adhesion complexes, but the advancing edges had strong FAK activity. Together, these data suggest that taut actin retraction fibers of similar nature are formed upon cell retraction in either cell division or cell migration. Furthermore, the absence of well-defined focal adhesions at their ends in

each case implies that their attachment to the substratum is likely only of low strength.

RhoA and motility

We next examined the underlying molecular mechanisms responsible for the tension in the actin retraction fibers. Since activated RhoA leads to the formation of stress fibers and is known to also play an important role in the retracting edge of migrating cells (19,20), we established a stable line expressing a CFP-YFP FRET-based RhoA biosensor (15). Although RhoA function has been implicated at the cleavage furrow during division and in cortical retraction and rigidity (10,21), there is increasing evidence for a broader involvement of Rho-GTPases in mitosis (22). Indeed, we observed the presence of activated RhoA along the cell cortex and partly along retraction fibers of the rounded cell during prometaphase (Fig. 4, *a* and *b*). Attempts to directly block RhoA activity resulted in inhibition of cell division, which prevented us from studying its role directly. However, we found that the inhibition of Rho-associated kinase (ROCK; using Y27632), which is downstream of RhoA, did not block cell division, but instead prevented efficient rounding of the mother cell (Fig. 4, *c* and *d*), confirming its essential role in efficient cortical retraction (10). It has been shown that there is no myosin in the retraction fibers (5), and hence we conclude that the tension in the retraction fibers is likely due to the opposing forces of cortical retraction and remnant substratum adhesion of the fibers.

To verify this hypothesis and understand its implications, we systematically explored the processes that modulate the above two forces and thus the tension and integrity of actin retraction fibers. First, we examined the effect of weaker substrate adhesion forces by exploring whether induction of increased motility upon metastatic transformation in HeLa cells would affect the division process in the same way that we observed in OSE cells. Specifically, we examined the effect of oncogenic Ras (23) in HeLa cells. We observed that GFP-tubulin cells coexpressing RasV12 exhibited markedly increased motility rates (Fig. 5, *a* and *c*). Of importance, these transformed cells no longer divided within the outline of the mother cell (Fig. 5, *b* and *d*, Movie S6, and Movie S7).

Conversely, we examined the effect of ROCK inhibition in highly motile cells as a means to alleviate the force of cortical retraction. We observed that ROCK inhibition by Y27632 of the highly motile OSE cells greatly reduced the number of cells that lost retraction fiber attachment to the substrate, and consequently these cells proceeded to divide within the outline of the interphase shape of the mother cell (Fig. 5, *e–g*, and Fig. S4). Together, these results suggest that if the force of cortical retraction overcomes the force of local substrate adhesion, there will be a loss of association with the ECM due to a loss of the local actin retraction fibers. If this loss

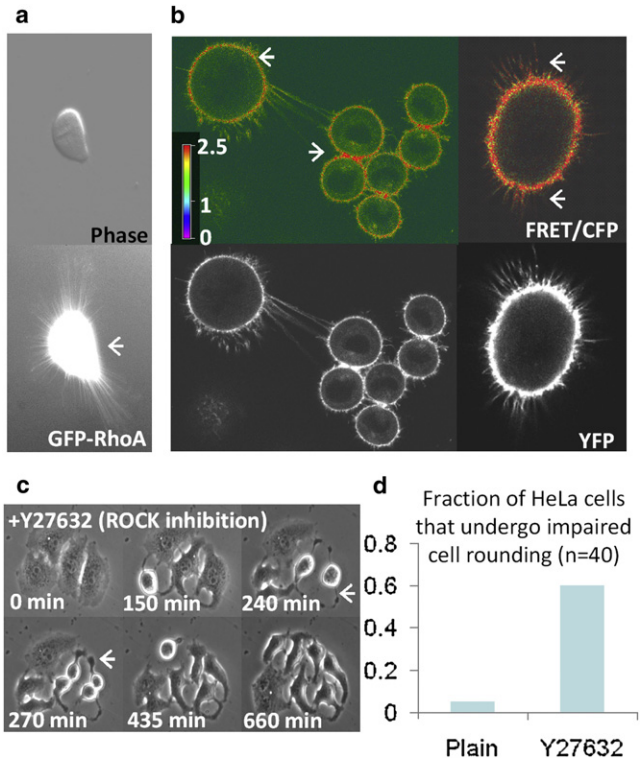

FIGURE 4 (*a*) Localization of RhoA, as visualized by a RhoA-GFP tagged construct, is observed throughout the mitotic cell body and along actin retraction fibers. (*b*) FRET data however indicates the presence of activated RhoA to be primarily along the mitotic cell cortex (see *arrows*) and partly also along retraction fibers. This is in addition to the known localization of activated RhoA along the cleavage furrow (see *arrows*) during cytokinesis. (*c* and *d*) Consistent with a critical role of RhoA in mitotic cell retraction, inhibition of ROCK activity prevents efficient rounding of the mother HeLa cell before division (data here were collected from >40 cell divisions in three independent experiments).

of adhesion is substantial, it can result in the detachment of whole mitotic cells during division (Fig. 1 *f*), whereas a partial loss will prevent conservation of the interphase outline of the mother cell by preventing daughter-cell spreading into those regions (Fig. 2 *b*). In conclusion, our data suggest that both the extent and strength of ECM association mediated by actin retraction fibers affect daughter-cell spreading, and that this is a function of the interplay between RhoA and the intrinsic motility of a cell (Fig. 5 *h*).

DISCUSSION

On the basis of our results, we propose that a balance between cortical retraction forces, which depend in part on RhoA activation, and substrate adhesion forces, which diminish with increasing motility rates, governs the integrity of mitotic actin retraction fibers and thus influences subsequent daughter-cell spreading (Fig. 6). Specifically, our data show that focal adhesions are lost from the ends of the retraction fibers formed upon cortical retraction (Fig. 4 *b*), and it is likely that they only weakly adhere to the

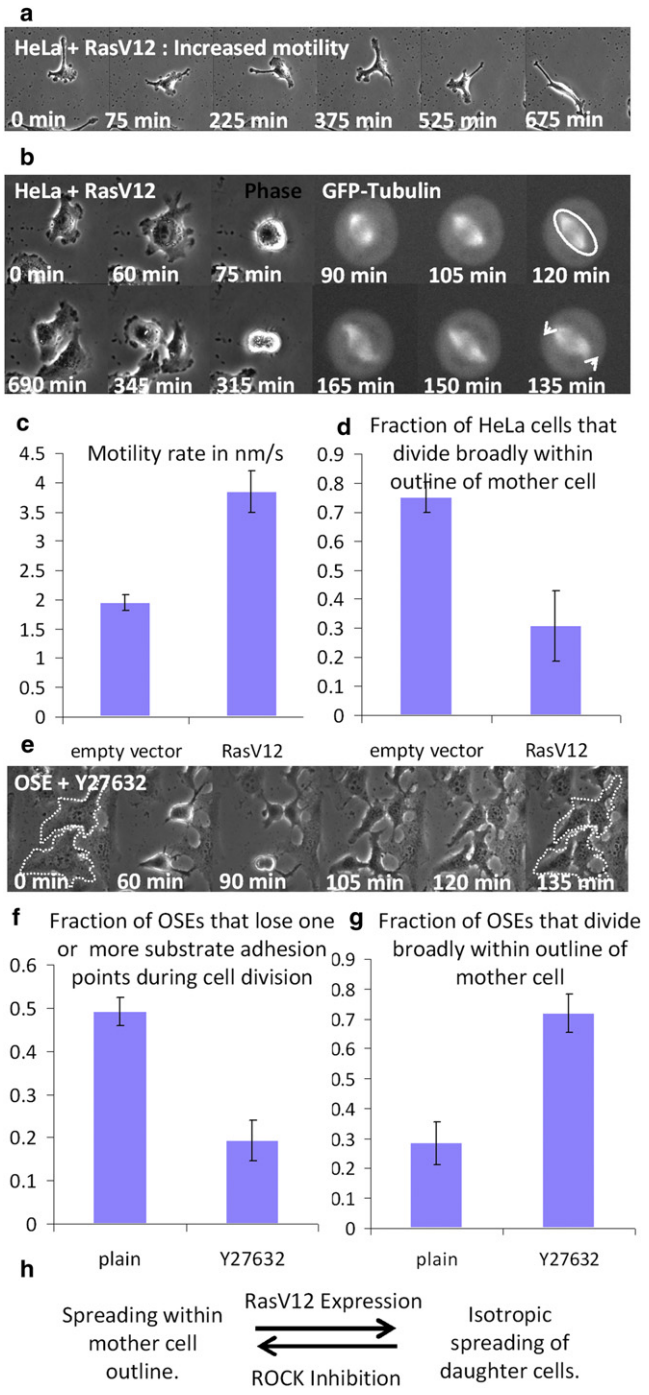

FIGURE 5 (*a–c*) Overexpression of RasV12 in HeLa cells results in acquisition of a highly motile phenotype (mean ± SE, $p < 0.005$). (*d*) Consistent with the observation in OSEs, a gain of motility phenotype in HeLa cells also results in a reduced degree of conservation of the mother cell outline by daughter cells upon division, i.e., these cells no longer divide within the outline of the mother cell (mean ± SE, $p = 0.0049$). (*e–g*) Conversely, ROCK inhibition in OSEs, dramatically reduces the fraction of motile cells that loose substrate adhesion during rounding (due to reduced detachment of retraction fibers), and consequently daughter cells now show an increased reutilization of the focal adhesion pattern of the interphase mother cell (mean ± SE, $p < 0.005$). (*h*) Summarizing, we show that it is possible to reversibly switch between the phenotypes of division within mother cell outline (HeLa) versus isotropic spreading (OSEs), using RasV12 overexpression in HeLa, which induces motility, and ROCK inhibition in OSEs, which prevents substrate detachment upon mitotic cell rounding.

substratum. Thus, if the force of cortical retraction exceeds this remnant adhesion strength, the corresponding retraction fiber will break its contact to the surface and cease to serve as a guide for subsequent daughter-cell spreading. This can happen either for highly motile cells, which have a reduced association and adhesion with the substrate as compared to less motile cells (14), or when the force of cortical retraction is large, such as upon RhoA overexpression, which has been shown to lead to mitosis-associated detachment of cells from epithelial cell layers (24). The latter is further supported by our observation that activated RhoA is localized along the cell cortex during mitosis and thus likely controls cortical retraction forces through downstream effectors such as ROCK. Consistent with this, we observe impaired cell rounding upon ROCK inhibition, which also serves as a potential strategy to mitigate retraction forces in motile cells to balance their reduced substrate adhesion forces (Fig. 5, *e–g*). Indeed, we find that in such a scenario, motile cells upon division tend to better reutilize their mother-cell adhesion footprint. Focal adhesion dynamics during cell division may share similar biochemical steps to those in fibronectin activated cell spreading and adhesion (25) or wound-healing migration patterns. Responses to local changes in cellular architecture during the course of division may also contribute to this process and merit further study (26).

The balance of forces during mitosis also has implications for cancer metastasis. We show that HeLa cells typically divide within the outline of the mother cell, and in fact, using patterned fibronectin islands of subcellular dimension that fix the locations of the focal adhesions of the mother cell, we further confirmed that daughter cells precisely reoccupy the key focal adhesion contacts of the mother cell. However, transformation of these cells through RasV12 overexpression increases their motility and results in a scattered growth of the cells that cease to show the typical epithelial morphology of the parental HeLa cells. Furthermore, the daughter cells now spread isotropically and cease to divide within the mother-cell outline. This is consistent with the implication of RhoA in this process, since Rac is known to antagonize Rho activity (27), and consequently sustained oncogenic Ras activity (RasV12 overexpression) can downregulate Rac activity and lead to an up-regulation of Rho activity (27,28). Together, our results showing the interplay and overlap of genetic and ECM cues have important implications for regulated tissue growth, since they imply that certain cell types must retain information about their shape and position, and that impairment of this process (either genetic or ECM-based) can lead to formation of a disorganized mass of cells or even dissemination of daughter cells. This is dramatically illustrated by

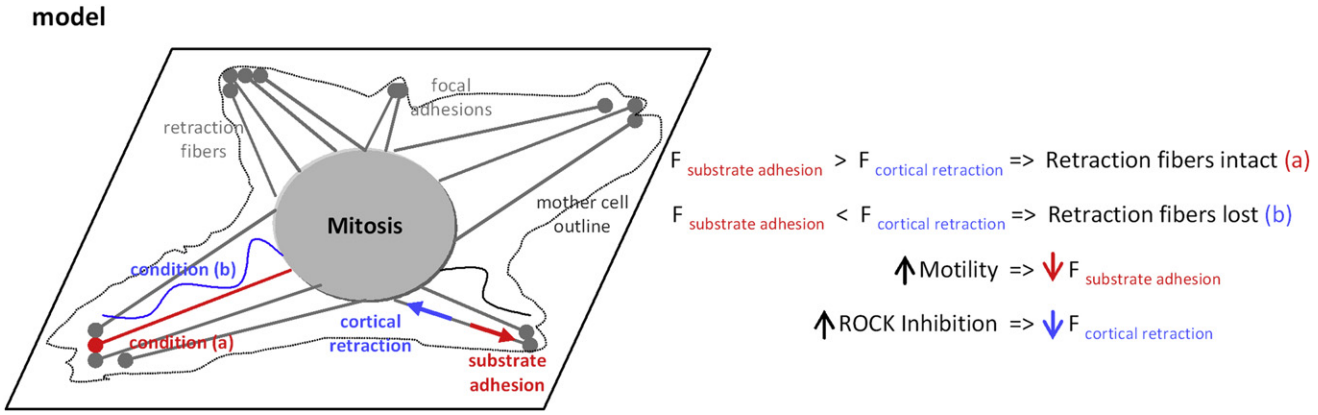

FIGURE 6 Our results suggest a model in which a balance between cortical retraction forces, which depend in part on RhoA activation, and substrate adhesion forces, which diminish with increasing motility rates, governs the integrity of mitotic actin retraction fibers and thus influences subsequent daughter-cell spreading. As highlighted, if the force of cortical retraction exceeds the strength of substrate adhesion, there will be a loss of a corresponding mitotic retraction fiber, and daughter cells will be prevented from spreading into those regions. Furthermore, appropriate modulation of these forces, as indicated, can be used to partially rescue or recapitulate these phenotypes.

the observation that a migrating cell can often disrupt the efficacious division of a neighboring cell if it invades the latter's local ECM (Fig. S1 *d* and Movie S8). Overall, these findings lead us to hypothesize that the more motile subpopulations of cells in a tissue may preferentially disseminate daughter cells during mitosis.

Taken together, our results provide experimental evidence for a model supporting the role of ECM cues in cell division modulated by the interplay of RhoA and intrinsic motility. These interactions influence the orientation of the mitotic spindle and consequently the axis of cell division, as well as processes that govern both cell rounding before division and daughter-cell spreading postmitosis. Furthermore, they play a critical role in maintaining the cell's positional integrity in a niche, and thus provide insight into the mechanisms of organized tissue growth and metastasis.

SUPPORTING MATERIAL

Four figures and eight movies are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)01254-3.

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