Actomyosin Pulls to Advance the Nucleus in a Migrating Tissue Cell

Jun Wu, Ian A. Kent, Nandini Shekhar, T. J. Chancellor, Agnes Mendonca, Richard B. Dickinson, and Tanmay P. Lele*
Department of Chemical Engineering, University of Florida, Gainesville, Florida

ABSTRACT The cytoskeletal forces involved in translocating the nucleus in a migrating tissue cell remain unresolved. Previous studies have variously implicated actomyosin-generated pushing or pulling forces on the nucleus, as well as pulling by nucleus-bound microtubule motors. We found that the nucleus in an isolated migrating cell can move forward without any trailing-edge detachment. When a new lamellipodium was triggered with photoactivation of Rac1, the nucleus moved toward the new lamellipodium. This forward motion required both nuclear-cytoskeletal linkages and myosin activity. Apical or basal actomyosin bundles were found not to translate with the nucleus. Although microtubules dampen fluctuations in nuclear position, they are not required for forward translocation of the nucleus during cell migration. Trailing-edge detachment and pulling with a microneedle produced motion and deformation of the nucleus suggestive of a mechanical coupling between the nucleus and the trailing edge. Significantly, decoupling the nucleus from the cytoskeleton with KASH overexpression greatly decreased the frequency of trailing-edge detachment. Collectively, these results explain how the nucleus is moved in a crawling fibroblast and raise the possibility that forces could be transmitted from the front to the back of the cell through the nucleus.

INTRODUCTION

The nucleus is the largest subcellular organelle of the cell and performs diverse functions, including genome organization, gene regulation, regulation of nucleocytoplasmic transport, and nuclear signaling. Precise positioning of the nucleus is a necessary step during cell and tissue functions such as cell polarization (1), cell migration (2,3), cell division (4,5), and development (6–8). Defects in positioning of the nucleus can lead to a host of human disorders (9,10). The mechanisms by which nuclear position is established in cells and tissues are of great interest. The forces that act to position the nucleus are typically considered to be from two sources: actomyosin contraction (2,11) and the activity of nuclear-linked microtubule motors (12–17).

Models to explain how nuclear positions are established in the cell fall into three classes. In one class, the nucleus is primarily assumed to be under tension from discrete tensile actomyosin cables that are connected to the nuclear surface (18). In this model, actomyosin forces pull on the nucleus symmetrically, resulting in nuclear deformation (19,20). Such a model has been used to explain how mechanical forces at the cell surface adhesion receptors could be channeled along cytoskeletal filaments to the nuclear surface (18). Unlike the static picture, which is suggested in the model in which the nucleus is hardwired to the cytoskeleton, in crawling cells, both F-actin and microtubule networks are continuously remodeled (21) throughout the cyclical process of protrusion, adhesion, and detachment/retraction of the trailing edge. During this cell-locomotion cycle, the nucleus advances with the cell to remain roughly at the cell center, pointing to a dynamic force balance on the nucleus. If this model is also valid for a migrating cell, then it would suggest a predominant role for tensile actomyosin forces in positioning the nucleus near the cell center. This view is supported by a recent paper (22) that explained oscillatory motion of nuclei in cells using tensile actomyosin forces.

The second, more recently proposed class of models offers a different mechanical explanation for nuclear positioning and establishment of shape based on shear or compression forces. For example, previous studies proposed that the nucleus is primarily pushed into position away from the leading edge by retrograde flow of actomyosin stress fibers on the apical surface of the nucleus (23,24). A recent paper also suggested that stress fibers compress the nucleus in elongated cells laterally, causing nuclear elongation (25). It has also been proposed that the nucleus is pushed forward during crawling by actomyosin squeezing forces in the (detached) trailing edge (6,7).

The third class of models seeks to explain how nuclei are positioned by translocation along microtubule tracks through the motoring activity of nuclear-envelope-bound microtubule motors (26–28). In muscle cell development, for example, the regular positioning of nuclei requires microtubules and the activity of both kinesin-1 and dynein (17). In static and migrating fibroblasts, dynein activity is necessary for inducing nuclear rotations (12,16). Bidirectional movements of nuclei in *Caenorhabditis elegans* embryos (29) and oscillatory nuclear motion between cell

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*Correspondence: tlele@che.ufl.edu

Jun Wu and Ian A. Kent contributed equally to this work.

Jun Wu's present address is Department of Chemical and Biomolecular Engineering, University of Illinois, Urbana-Champaign, Illinois

Nandini Shekhar's present address is Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts

Agnes Mendonca's present address is School of Chemical Engineering, Purdue University, West Lafayette, Indiana

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poles during meiotic prophase in *Schizosaccharomyces pombe* (30) are both driven by dynein. Microtubule-motor-based forces are therefore a key component of the nuclear force balance and may even be the predominant mechanism for determining nuclear position in certain cell types.

In this work, we determined the dominant mechanical forces that position the nucleus in a crawling NIH 3T3 fibroblast by directly manipulating actomyosin and microtubule-based force generators at the front and back of the cell. When a new lamellipodium was triggered with photoactivation of Rac1, the nucleus moved toward the new lamellipodium in a myosin-dependent manner. This finding was unexpected, as the nucleus typically is expected to be pushed back by retrograde flow of actomyosin from the leading edge (1). The motion was independent of microtubule motor forces. The rear edge of the nucleus was found to be mechanically coupled to the trailing edge, i.e., tensile force was transmitted from the substratum to the nucleus. Our results suggest that actomyosin pulling forces, rather than pushing forces, are the dominant forces that translocate the nucleus during cell migration.

MATERIALS AND METHODS

Cell culture, plasmids and transfection, and drug treatment

NIH 3T3 fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM; Mediatech, Manassas, VA) with 10% donor bovine serum (Gibco, Grand Island, NY). For microscopy, cells were cultured on glass-bottomed dishes (MatTek, Ashland, TX) coated with 5 μg/ml fibronectin (BD Biocoat, Franklin Lakes, NJ) at 4°C overnight. For photoactivation experiments, cells were serum starved for 2 days in DMEM with 1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO).

Transient transfection of plasmids into cells was performed with Lipofectamine 2000 transfection reagent (Life Technologies/Invitrogen, Carlsbad, CA). The following constructs were used in this study: mCherry-PA-Rac1 (Addgene plasmid 22027, Addgene, Cambridge, MA), GFP actin (a gift from Prof. D.E. Ingber, Harvard University), YFP-γ-tubulin prepared from the MBA-91 AfCS set of subcellular localization markers (ATCC, Manassas, VA), DsRed-CC1 to inhibit dynein activity as previously described (16,31) (provided by Prof. Trina A. Schroer, Johns Hopkins University), EGFP-KASH4 to disrupt the LINC complex as previously described (14) (provided by Prof. Kyle Roux, Sanford Children's Health Research Center), and LifeAct-TagRFP (Ibidi, Verona, WI).

Microtubules were disrupted by treating cells with nocodazole (Sigma-Aldrich) at a final concentration of 1.6 μM for >1 h before Rac1 photoactivation. Y-27632 (EMD Millipore, Billerica, MA) or ML-7 (Sigma-Aldrich) was added to cells for myosin inhibition for >1 h before Rac1 photoactivation at concentrations of 10 μM and 25 μM, respectively.

Time-Lapse Imaging and Analysis

Time-lapse imaging was performed on a Nikon TE2000 inverted fluorescent microscope with a 40X/1.45 NA oil immersion objective and CCD camera (CoolSNAP, HQ^2; Photometrics, Tucson, AZ). During microscopy, cells were maintained at 37°C in a temperature-, CO_2-, and humidity-controlled environmental chamber. Time-lapse images of actin stress fibers were deconvolved using Nikon NIS-Elements software.

Fixation and Immunocytochemistry

For determination of phospho-myosin distribution in migrating cells, 3T3 cells were simultaneously fixed and permeabilized for 20 min in 4% (m/v) paraformaldehyde + 0.5% (v/v) Triton X-100 in PBS prewarmed to 37°C. The cells were then rinsed several times with PBS and blocked in 1% (m/v) BSA (Sigma-Aldrich) in PBS for 30 min. A 1% BSA solution was also used to dilute antibodies and dyes in later steps. Cells were incubated for 1 h at room temperature in a 1:50 dilution of Rb-anti-phospho-myosin light chain 2 (Ser19, No. 3671; Cell Signaling Technology, Danvers, MA) (32), rinsed with PBS, and then incubated with a 1:500 dilution of Alexa Fluor 488 goat anti-rabbit IgG antibody (Life Technologies). To stain F-actin, cells were incubated for 20 min in 1:200 Alexa Fluor 594 phalloidin at room temperature. Finally, the cells were incubated at room temperature in Hoechst 33342 at 1:200 dilution for 20 min to visualize the nucleus.

Confocal Microscopy and Photoactivation

Cells were imaged on a Leica SP5 DM6000 confocal microscope equipped with a 63X/1.4 NA oil immersion objective. For photoactivation, a region in between the nucleus and the edge of a cell, which is approximately the size of the nucleus, was chosen using the region of interest (ROI) function. Photoactivation was achieved with a 488 nm Argon laser applied at 1% power every 10 s. Cells were maintained at 37°C in a temperature-, CO_2-, and humidity-controlled environmental chamber during microscopy. All photoactivation experiments were performed for 30 min to be consistent with the nuclear tracking measurements.

Image Analysis

Images of migrating cells were processed with ImageJ software (NIH; contrast enhancement) and imported into MATLAB (The MathWorks, Natick, MA) to track the nuclear centroid and the contour of cells using custom-made programs. Image series from the photoactivation experiment were imported into MATLAB and the custom program was used for nuclear position tracking. After the positions of nuclear centroids in the photoactivation experiment were measured, the coordinates were rotated as shown in Fig. S1 in the Supporting Material. The vector pointing from nuclear centroid at time $t = 0$ to the photoactivation center was used as the $\theta = 0$ axis in polar coordinates. All of the trajectories were rotated following this rule. The directional movements were then calculated as the projected distance of the trajectories on the $\theta = 0$ axis.

The variance of nuclear position V at time k was calculated using the following formula:

$$V(k) = \sum_{i=1}^{N} \left\{ (x(i,k) - \bar{x}(k))^2 + (y(i,k) - \bar{y}(k))^2 \right\} / N$$

where $x(i,k)$ and $y(i,k)$ are the x and y coordinates of the nucleus in trajectory i at time k, and $\bar{x}$ and $\bar{y}$ are the mean x, y coordinates at time k.

Trailing-Edge Detachment

An Eppendorf Femtojet microinjection system (Eppendorf North America, Hauppauge, NY) was used to lower a micropipette (with a 0.5 μm diameter tip) onto the surface of the dish 250 μm from the cell. The needle was then lowered slowly, bending the main shaft of the needle and translating the tip across the surface of the glass bottomed dish until the tip slid underneath the tail of the cell. The needle was then translated toward the trailing edge. After a slight translation, the needle was raised through a distance of 3–5 μm. This was repeated until the trailing edge was removed. For repulling experiments, the tip of the needle was carefully lowered on top of the

previously released trailing edge and pressed against the glass surface. The tip was then translated away to reapply tension to the cell.

RESULTS

Forward motion of the nucleus can occur without requiring trailing-edge detachment

The fact that the nucleus is mechanically integrated with the actomyosin cytoskeleton raised a key question: how could the nucleus be positioned in a crawling cell where the actomyosin cytoskeleton is continuously remodeled? To determine the dominant cytoskeletal forces that translocate the nucleus in NIH 3T3 fibroblasts, we first tracked motion in crawling cells with a clearly polarized morphology. When the trailing edge detached spontaneously, significant forward motion of the nucleus toward the leading edge occurred, as has been reported previously (33,34) (Movie S1). However, trailing-edge detachment was not necessary for forward motion of the nucleus. We observed many examples of persistent forward nuclear motion occurring as the leading lamella expanded with no detachment of the trailing edge (Fig. 1 *A*; Movie S2). In these cases, tracking the positions of the nucleus centroid, cell centroid, and trailing-edge positions revealed that significant forward motion of the nucleus typically accompanied forward motion of the cell centroid without any measurable motion of the trailing edge, as shown in Fig. 1, *B* and *C*. We conclude that forward motion of the nucleus correlates with cell centroid motion, but can occur without trailing-edge detachment or any large changes in the shape of the trailing edge.

What causes forward nuclear motion in the absence of any trailing-edge detachment? One hypothesis (21) is that the nucleus is pulled forward by actomyosin contraction occurring between the nucleus and the leading edge. To test this idea, we first stained phosphorylated nonmuscle myosin II and F-actin in migrating cells (Fig. 1, *D* and *E*). Active myosin was found distributed in punctate spots in the lamella

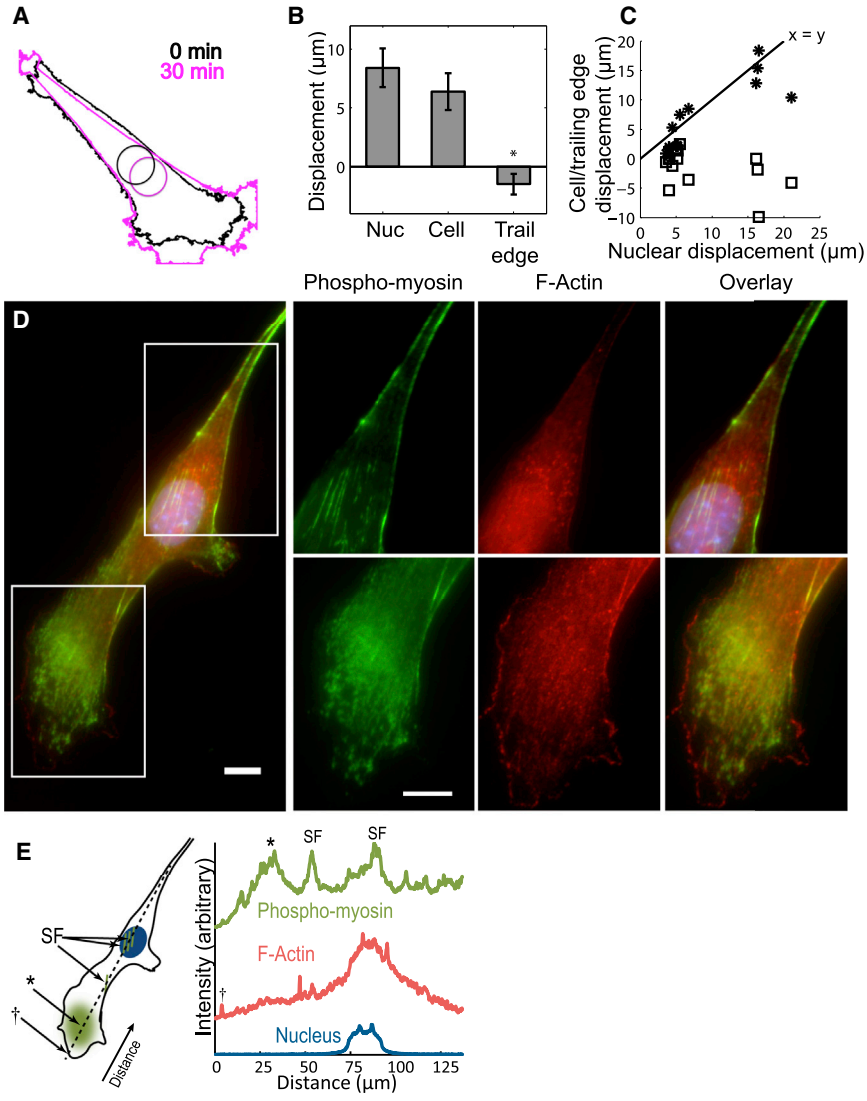

FIGURE 1 Actomyosin pulls on the nucleus toward the leading edge. (*A*) Superposition of the cell outline at 0 and 30 min, showing that the nucleus moves while the trailing edge remains intact. (*B*) Comparison of mean movement of the nucleus, cell centroid, and trailing edge in 30 min shows that the nucleus and cell centroid moved similar distances, but the trailing edge did not move appreciably ($n = 14$). Error bars indicate standard error of the mean (SEM); $*p < 0.01$. (*C*) Nuclear movement is correlated with cell centroid movement (*stars*, correlation coefficient $R = 0.8569$) but uncorrelated with trailing-edge movement (*squares*, $R = -0.4921$; $n = 14$). (*D*) A representative migrating 3T3 cell that was fixed and stained for phospho-myosin light chain 2 (*green*), F-actin (*red*), and DNA (*blue*). The cell is migrating toward the bottom left of the image. Magnified images of the trailing and leading edges qualitatively show an accumulation of phosphorylated myosin at the lamella and its relative absence behind the nucleus. Scale bars: 10 μm. (*E*) A line was drawn through the cell such that the different stain intensities could be compared throughout the length of the cell. Phospho-myosin stain intensity exhibits peaks at the lamella and at actomyosin stress fibers. To see this figure in color, go online.

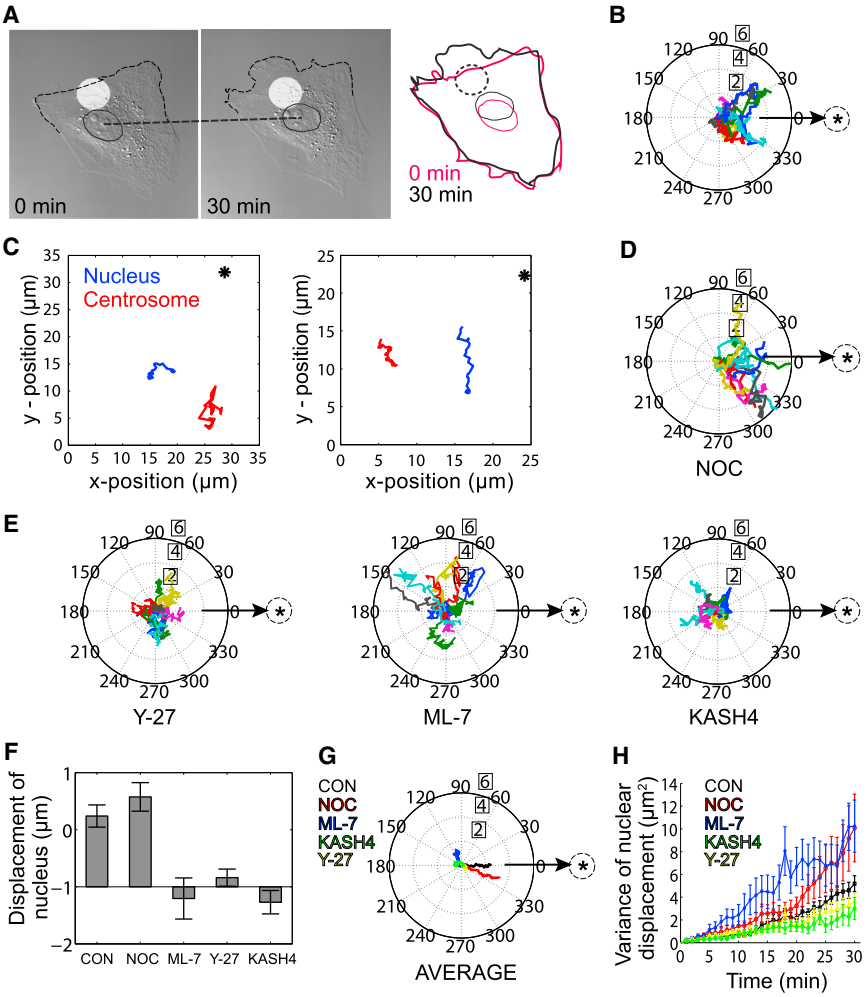

FIGURE 2 Photoactivation of Rac1 to induce lamellipodium formation causes directional bias in nuclear translation. (*A*) Images from a photoactivation experiment showing the nucleus (*outlined with solid line*) moving toward the photoactivation site (*bright circles*); the newly created leading edge is indicated with dashed curves. Also shown is a superposition of the cell and nuclear outlines at 0 and 30 min. Scale bar: 10 μm. (*B*) Trajectories of the nucleus upon photoactivation ($n = 11$; angles are in degrees; * represents the photoactivation center, so the nucleus-photoactivation center axis is oriented initially along the positive x axis); all trajectories start at the center. Boxed numbers are in microns. (*C*) Representative trajectories of the nucleus and centrosome; * indicates photoactivated spot. (*D*) Nuclear trajectories in cells treated with nocodazole (NOC), a microtubule disruptor ($n = 11$). (*E*) Nuclear trajectories in cells treated with ROCK inhibitor Y-27 ($n = 14$), MLCK inhibitor ML-7 ($n = 10$), and KASH4-expressing cells ($n = 11$). (*F*) Mean nuclear displacement projected along the positive x axis (CON, control). (*G*) Mean nuclear displacements. (*H*) Variance of the nuclear displacements relative to the mean displacements. To see this figure in color, go online.

and in actomyosin bundles, while F-actin was visible at the leading edge as well as in actomyosin bundles throughout the cell. In the trailing edge, phospho-myosin staining was present primarily in actomyosin bundles. This supports the concept proposed by Lauffenburger and Horwitz (21) that localized actomyosin contraction in the region between the lamella and the nucleus could pull the nucleus forward.

Actomyosin contraction between the leading edge and the nucleus pulls the nucleus forward

To test this possibility further, we adapted the recently introduced Rac1 photoactivation assay (35–37). The aim of this approach is to trigger local polymerization of F-actin and create new lamellipodia, which should cause an increase in contractile forces owing to newly formed actomyosin between the nucleus and the leading edge (21,38). Rac1 photoactivation caused new lamella formation and a clear increase in the local F-actin concentration (Fig. S2; Movies S3 and S4).

Upon local creation of a new leading lamella with Rac1 photoactivation (Fig. 2 *A*; the *white circle* indicates the photoactivated spot), the nucleus was observed to move persistently toward the direction of the new leading edge (Fig. 2, *A* and *B*; Movie S4). As shown in Fig. 2 *B*, the nucleus trajectories, although meandering, consistently drifted in the direction of the photoactivated spot (direction of the positive x axis in Fig. 2 *B*). One plausible explanation for the directional motion of the nucleus toward the photoactivated lamellipodium is that the centrosome repositions itself (as it tracks the cell centroid) and carries the nucleus with it through the action of nucleus-linked microtubule motors such as dynein or kinesin (26–28). In fact, the trajectories of the nucleus did correlate with centrosomal trajectories (both moved in the general direction of the newly created lamellipodium; Fig. 2 *C*). However, depolymerization of microtubules with nocodazole did not eliminate the directional motion of the nucleus (Figs. 2 *D*, S3 and, S4A; Movie S5), indicating that the nucleus was not being repositioned by the microtubule network. Consistent with this, we found that the nucleus could move forward without requiring trailing-edge detachment in crawling cells expressing CC1, a competitive inhibitor of dynein. The motion was similar to that in control cells because the nucleus tracked the cell centroid (Fig. S5). Inhibition of myosin activity by treatment with ML-7, a myosin light-chain kinase

(MLCK) inhibitor, or Y-27632, a rho-kinase (ROCK) inhibitor, and disruption of the LINC complex by overexpression of the KASH4 domain each eliminated the directional motion of the nucleus toward the photoactivated spot (Figs. 2, *E* and *F*, S4, B–D; Movies S6, S7, and S8; as is evident in Fig. S4, Rac photoactivation was able to produce clear lamellipodia in myosin-inhibited, microtubule-disrupted, and KASH4-expressing cells). We further analyzed the trajectories for each condition by calculating the mean nucleus displacement (Fig. 2, *F* and *G*) and the variance of the displacement relative to the mean (Fig. 2 *H*). Only control and nocodazole-treated cells showed significant nuclear displacement toward the photoactivated spot; KASH4-overexpression and ML-7- and Y-27632-treated cells showed essentially zero mean displacements. Nocodazole-treated cells had significantly higher variance in nuclear displacement relative to control cells, suggesting that microtubules interactions may dampen fluctuations in the nuclear trajectories. ML-7-treated cells also displayed a higher variance for reasons that are unclear.

Mechanical coupling between the nucleus and the trailing edge

We next mechanically detached the trailing edge by translating a micropipette tip under the trailing edge (Fig. 3 *A*). Trailing-edge detachment caused the nucleus to move toward the leading edge (Fig. 3 *A*; Movie S9). This motion could be interpreted as being due to pushing forces generated by actomyosin contraction that squeezes the trailing edge contents, a dissipation of tensile forces on the trailing surface of the nucleus, or some combination of both. The forward nuclear motion was also accompanied by a change in the shape of the nucleus. To quantify the motion and shape changes, we tracked leading and trailing surfaces of the nucleus on trailing-edge detachment. Both nuclear leading and trailing edges of control cells moved forward when the trailing edge was detached, but this motion was significantly decreased in KASH4-overexpressing cells (Fig. 3, *B* and *C*; Movie S10). The aspect ratio of the nucleus (measured as nucleus width in the direction of the cell's long axis divided by the perpendicular nucleus width) consistently decreased on trailing-edge detachment, reflecting a longitudinal flattening of the nucleus along the cell axis with time (Fig. 3 *D*). In contrast, the nucleus did not change shape significantly when the trailing edges of KASH4-overexpressing cells were detached (Fig. 3 *D*). Interestingly, when a detached trailing edge was again pulled by a micropipette and reextended to its original length (Fig. 3 *E*), the nucleus immediately recovered its original shape. Fig. 3 *F* shows that the motion of the nucleus

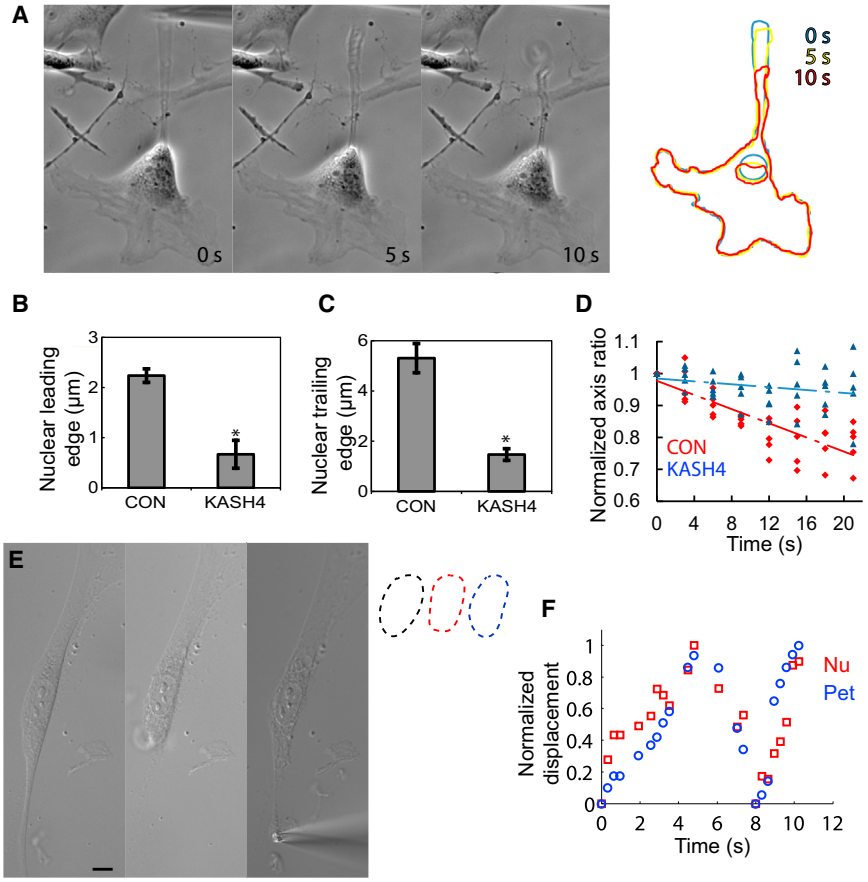

FIGURE 3 Micromanipulation experiments show that the nucleus is under tension between the leading edge and trailing edge. (*A*) Images show the release of the trailing edge of the cell by micromanipulation with a micropipette. Scale bar: 10 μm. Superposition of cell and nuclear outlines at 0, 5 and 10 s shows the forward motion and deformation of the nucleus. (*B* and *C*) Quantification of the forward movement reveals that both the leading (*B*) and trailing (*C*) edges of the nucleus traveled farther in control cells than in KASH4-expressing cells. Error bars indicate SEM; $*p < 0.01$. (*D*) Upon trailing-edge detachment, the nucleus progressively flattened along the axis, joining the nucleus and the trailing edge in control cells ($n = 5$), but not in KASH4 transfected cells ($n = 5$). The normalized axis ratio is the long axis over the short axis of the nucleus normalized by its value before detachment. (*E*) Pulling on the detached trailing edge of the cell results in nuclear movement in the direction of the pull and restoration of elongated nuclear shape. Nuclear position and shape are indicated in the dashed outlines. Scale bar: 10 μm. (*F*) Normalized displacements of the nucleus (Nu) tightly track displacements in the micropipette (Pet) attached to the detached trailing edge. To see this figure in color, go online.

correlates closely with the motion of the micropipette that is attached to the trailing edge, indicating transmission of force between the nucleus and the trailing edge. Together, these results suggest that the nucleus shape is governed by tensile actomyosin structures, because although actomyosin squeezing forces could feasibly move the nucleus forward upon detachment, re-elongation of the nucleus due to trailing-edge extension cannot be explained by pushing forces in the reverse direction.

Apical actin bundles align lengthwise in migrating cells and translate orthogonal to the direction of nuclear motion

We next asked whether the forces from actomyosin contraction that pull the nucleus forward could be transmitted to the nuclear surface by translating apical actomyosin bundles. This was motivated by previous observations that in wounded monolayers, the nucleus moves backward away from the wounded edge, and this movement correlates with the motion of actomyosin bundles orthogonal to the direction of nuclear motion (1,24). Rearward translation of actomyosin bundles is thought to shear the top surface of the nucleus through connections called TAN lines and carry it.

We imaged migrating fixed 3T3 fibroblasts stained with phalloidin as well as live cells expressing GFP-actin or LifeAct-TagRFP. Apical stress fibers, when visible, were oriented along the length of the cell, as were basal stress fibers in general (Fig. 4, *A–C*). The apical stress fibers were often dynamic, translating laterally across the width of the cell (Fig. 4, *E* and *F*; Movies S11, S12, S13, and S14). We did not observe apical fibers oriented orthogonal to the cell axis that translated with the direction of the

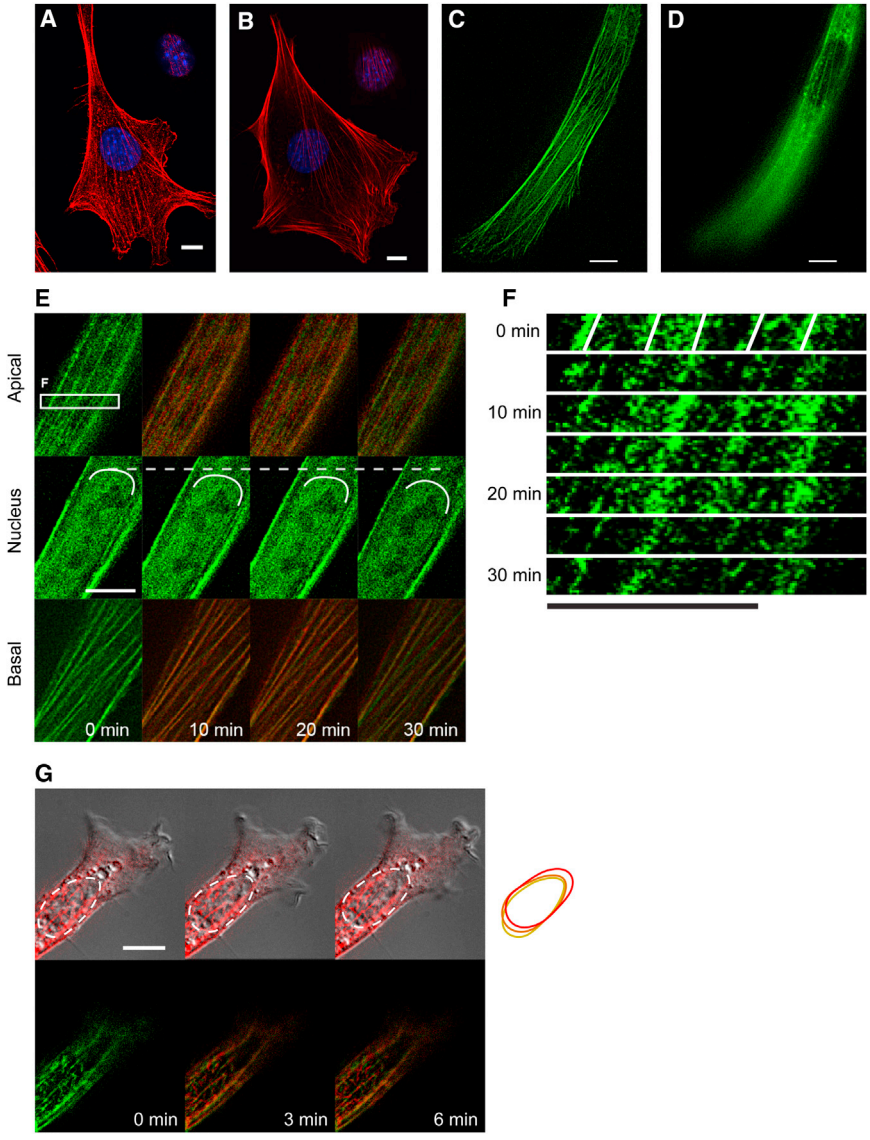

FIGURE 4 Actin dynamics in migrating 3T3 fibroblasts. (*A* and *B*) Basal (*A*) and apical (*B*, *inset*) stress fibers in fixed 3T3 fibroblasts. Stress fibers above and below the nucleus are generally oriented lengthwise in the cell. (*C* and *D*) Basal (*C*) and apical (*D*) stress fibers in a migrating 3T3 fibroblast are transiently expressed. Both are oriented lengthwise. (*E*) Displacement of stress fibers and forward motion of the nucleus in cell C over 30 min. Displacements of apical and basal stress fibers are denoted by a red overlay over a green image taken at $t = 0$ min. Overlap between the current time point and $t = 0$ min is orange in color. It is clear that apical stress fibers displace at a faster rate than basal stress fibers. (*F*) Lateral motion of apical actomyosin stress fibers in cell C over 30 min. Images are cropped as shown in the top-left panel of *E*. The observation that apical stress fibers move laterally over the nucleus suggests that forward motion of the nucleus is not caused by apical stress fibers. Conversely, basal stress fibers displace much more slowly than the nucleus of a migrating cell, so they likely do not contribute to the nucleus's forward motion either. (*G*) Forward motion of the nucleus in a cell expressing LifeAct-TagRFP. Forward motion occurs even when apical stress fibers are relatively stationary. Differences from time zero are shown as in *E*. All scale bars: 10 μm. To see this figure in color, go online.

nuclear motion. It was possible to observe short periods of time (<10 min) during which the apical stress fibers were relatively static as the nucleus continued to move forward (Fig. 4 *G*).

KASH expression decreases the detachment frequency of the trailing edge

If the pulling forces acting upon the front of the nucleus are balanced by corresponding tensile forces at the back, then it is possible that the nucleus transmits forces long range (i.e., from the front to the back of the cell) to potentially detach the trailing edge. Consistent with this concept, we found that the trailing-edge detachment frequency was higher in control cells than in KASH4-overexpressing cells (Fig. 5 *A*). Although nuclear movement was still highly correlated with cell centroid movement (Fig. 5, *B* and *C*), as observed in control cells, KASH4-expressing cells moved forward through a sliding of the trailing edge rather than by detachment (Fig. 5 *E*, compare with control cell in Fig. 5 *D*). Thus, the nucleus may well act as a long-range transmitter of forces between the front and back of the cell to enable normal cell migration.

DISCUSSION

The mechanism by which the nucleus is positioned in cells and tissues is of emerging interest (1–5). In a migrating NIH 3T3 fibroblast, the nucleus is under a dynamic force balance that reflects the dynamic remodeling of the cytoskeleton. For isolated cells crawling on a two-dimensional substrate, a common view is that the nucleus is pushed forward by actomyosin squeezing forces in the (detached) trailing edge (6,7). In this work, we showed that the nucleus could move forward in the absence of trailing-edge detachment in isolated NIH 3T3 fibroblasts with a polarized morphology. In these instances, the leading lamella changed in size, leading to a forward translation of the cell centroid (the trailing edge was essentially stationary).

To understand the forces that position the nucleus in the absence of any trailing-edge detachment, we used a Rac1 photoactivation assay to locally trigger the formation of lamellipodia. As expected, the nucleus translated toward the site of new lamellipodia formation (the cell shape was essentially constant elsewhere). Although the motion of the nucleus correlated with the motion of the centrosome, it occurred even when microtubules were depolymerized, and it was completely abrogated upon myosin inhibition. Although microtubules were dispensable for this motion, we found that the nuclear trajectories in nocodazole-treated cells contained significantly more deviations from the straight path toward photoactivation spot. Thus, microtubule association with the nucleus (through molecular motors) may be important for nuclear positioning because it can damp nuclear fluctuations (such as those caused due to the formation of new lamellipodia at the cell edge).

Upon generation of a new lamellipodium, there was an increase in the local F-actin concentration (Fig. S2), which likely led to an increase in contractile forces owing to newly formed actomyosin between the nucleus and the leading edge (9,10). The increased contraction pulled the nucleus toward the newly formed lamellipodium. This finding is in apparent contradiction to previous studies indicating that the nucleus moves away from the leading edge in cells at the boundary of a scratch wound (1,24). However, a key difference is that in the previous studies, nuclear motion was observed in cells at the edge of a cell monolayer, where cell-to-cell pulling forces are relevant; these forces are

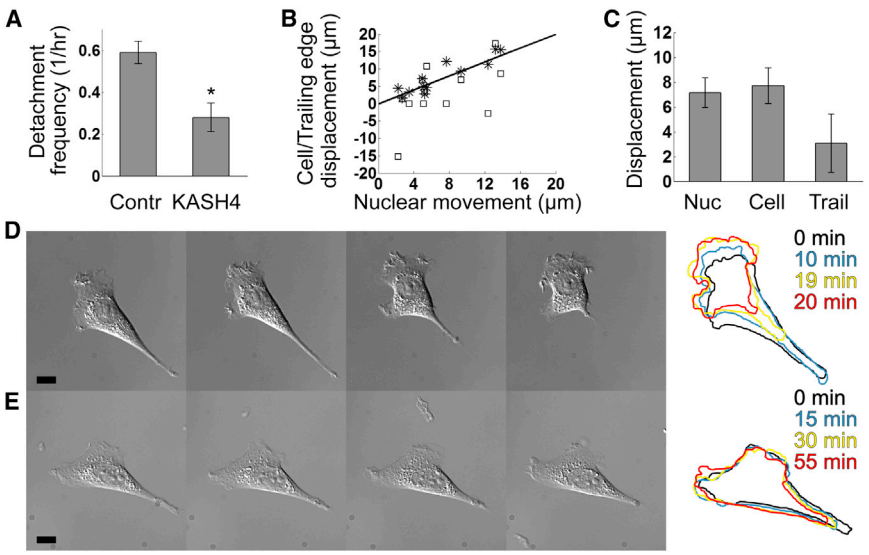

FIGURE 5 Effect of KASH on trailing-edge detachment. (*A*) Trailing-edge detachment frequency is much higher in control cells ($n = 24$) than in KASH4-transfected cells ($n = 20$). (*B*) Nuclear movement is highly correlated with cell centroid movement (*stars*, R = 0.9194) in KASH4-transfected cells ($n = 11$) and correlated with the trailing-edge movement (*squares*, R = 0.5254). The solid line is the y = x line. (*C*) Average movements of the nucleus and cell centroid in KASH4-transfected cells ($n = 11$) in 30 min show that they move similar distances. The trailing edge also moves forward. Error bars indicate SEM; $*p < 0.01$. (*D*) Images of the trailing-edge detachment during forward protrusion of an NIH 3T3 fibroblast and superposition of the cell outline at different time points. (*E*) Images of trailing-edge movement during forward protrusion of a KASH4-transfected cell and superposition of the cell outline at different time points. The trailing edge slides forward instead of detaching from the substrate (compare with outlines in *D*). To see this figure in color, go online.

absent in isolated cells. If these cell-cell pulling forces are transmitted to the nucleus and exceed the pulling force from the new leading edge, a net rearward motion of the nucleus should occur.

We found that apical and basal actin fibers align along the direction of the cell axis in isolated migrating 3T3 fibroblasts. In some cases, apical fibers moved laterally across the nucleus during cell migration. Forward nuclear motion still occurred even when apical stress fibers did not translate. We did not observe any apical fibers oriented perpendicular to the cell axis in these cells. It is possible that apical fibers differentially contract along their length and move the nucleus along through linkages maintained by the LINC complex. Another possibility is that the contraction between the leading edge and the nucleus could pull directly on the nucleus through attachments between the mesh-like F-actin network that pervades the cytoplasm and could be bound to the nucleus on the sides. Alternatively, contraction could pull on other nucleus-attached cytoskeletal elements, such as microtubules or intermediate filaments. Future studies focusing on the precise structures that connect and pull on the nucleus are needed to address this.

The forward pulling force on the nucleus may be balanced by actomyosin pulling forces on the nucleus exerted from the trailing edge, or by elastic forces originating in the actomyosin cytoskeleton. Consistent with this view, pulling and relaxing a detached trailing edge could produce reversible motion of the nucleus in a myosin-dependent manner, indicating transmission of mechanical force between the attachments at the trailing edge of the cell and the nuclear surface.

The model that emerges from these experiments is that the nucleus is subjected to a tug-of-war between anterior actomyosin contractile forces and posterior actomyosin forces originating either from actomyosin contraction or from elastic forces (Fig. 6 *A*) that simultaneously pull the nucleus forward toward the leading edge and rearward toward the trailing edge. Given that F-actin continuously polymerizes at the leading edge, there is a continuous source of actomyosin that can contract to pull on the nucleus. The trailing edge is relatively stable in shape (until it detaches), and hence it is reasonable to surmise that the tensile forces in the trailing edge are relatively constant in magnitude. Net forward motion of the nucleus would be predicted to occur when pulling forces at the front exceed those at the back (Fig. 6 *B*). The coordinated motion of a detached trailing edge with the nucleus appears to give the impression of the nucleus being pushed forward. Our results suggest that in this case, the nucleus is actually being pulled forward by actomyosin contraction from the front (Fig. 6 *C*). It could be that later in the process, pushing forces from the rear contribute a forward force on the nucleus as intracellular material is squeezed out of the detached trailing edge. However, our results indicate that the mechanism for nuclear centering during cell locomotion is likely a tug-of-war between pulling forces.

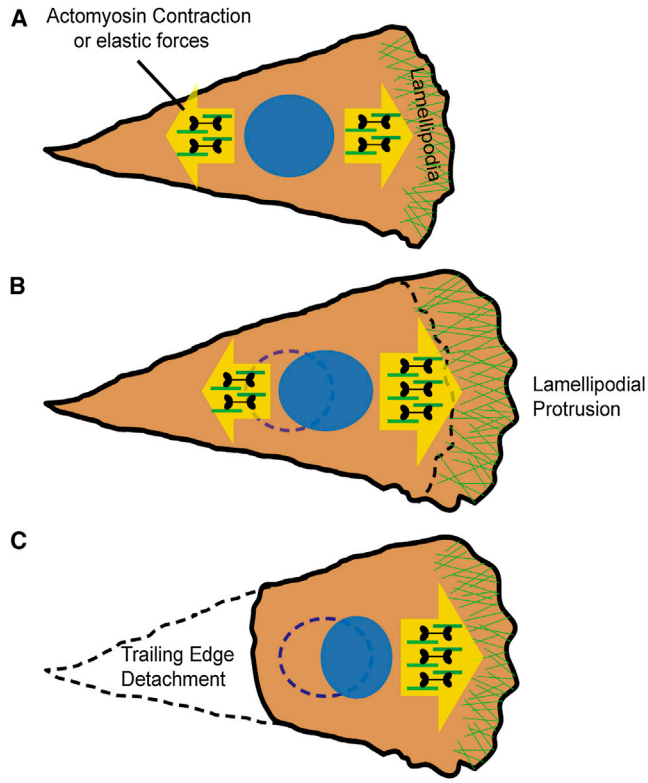

FIGURE 6 Tug-of-war model for nuclear positioning in a migrating tissue cell. (*A*) The nucleus (*blue*) is pulled simultaneously by both anterior actomyosin contractile forces and posterior actomyosin forces originating from either actomyosin contraction or elastic forces (*yellow arrows*). (*B*) Generation of new actomyosin from the leading edge increases the forward pulling force, causing an imbalance that moves the nucleus forward. (*C*) When the trailing edge detaches, the pulling force toward the rear becomes zero, causing the nucleus to quickly translate forward. To see this figure in color, go online.

Although nuclear positioning is clearly important for motion of the nucleus in the direction of the motile cell, it could well be that the positioning mechanism is crucial for force transmission to detach the trailing edge, which is required for normal cell motility (33). Recent studies have shown that LINC complex disruption reduces the persistence of cell migration (39,40). The actomyosin pulling-force balance suggests the intriguing possibility that LINC connections transmit contractile forces through the nucleus to detach the trailing edge. Such a model predicts that disrupting nucleus-actomyosin connections should decrease the frequency of trailing-edge detachment, resulting in abnormal migration. Our results in Fig. 5 support this possibility.

SUPPORTING MATERIAL

Five figures and 14 movies are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(13)05749-4.

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