Cytoplasmic Force Gradient in Migrating Adhesive Cells

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ABSTRACT Amoeboid movement is believed to involve a pressure gradient along the cell length, with contractions in the posterior region driving cytoplasmic streaming forward. However, a parallel mechanism has yet to be demonstrated in migrating adhesive cells. To probe the distribution of intracellular forces, we microinjected high molecular weight linear polyacrylamide (PAA) as a passive force sensor into migrating NIH3T3 fibroblasts. Injected PAA appeared as amorphous aggregates that underwent shape change and directional movement in response to differential forces exerted by the surrounding environment. PAA injected into the posterior region moved toward the front, whereas PAA in the anterior region never moved to the posterior region. This preferential forward movement was observed only in migrating cells with a defined polarity. Disruption of myosin II activity by blebbistatin inhibited the forward translocation of PAA while cell migration persisted in a disorganized fashion. These results suggest a myosin II-dependent force gradient in migrating cells, possibly as a result of differential cortical contractions between the anterior and posterior regions. This gradient may be responsible for the forward transport of cellular components and for maintaining the directionality during cell migration.

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Cell migration is critical for a wide range of physiological and pathological processes including embryogenesis, wound healing, cell-based immunity, and cancer invasion. Weakly adherent cells, including leukocytes and free-living amoebae, migrate by amoeboid movement, where protoplasmic flow is a prominent feature responsible for driving cytoplasmic materials toward the pseudopodia (1). As for fluid flow in vitro, this process is likely driven by a gradient of pressure, as a result of strong acto-myosin II-based cortical contractions in the posterior region coupled to the solation of cell cortex to form the cytoplasmic stream (1).

For adherent cells such as cultured fibroblasts, bulk cytoplasmic flow has never been reported due to the extensive tethering of visible organelles, whereas the cytoplasm somehow manages to move en mass during cell migration. Although intracellular pressure has been measured with an electrode (2), it is much more difficult to detect a spatial gradient. To address this question, we have used high molecular weight linear polyacrylamide (PAA) as novel pressure sensors. The neutral, heavily hydrated and inert properties of PAA lead to its general lack of binding with proteins and to its wide applications in denaturing and nondenaturing gel electrophoresis. These properties also made PAA an ideal material for sensing mechanical forces in the cytoplasm. We microinjected long (molecular weight >600,000) linear PAA at 5 mg/ml into the perinuclear region of NIH3T3 fibroblasts, either anterior or posterior to the nucleus relative to the direction of migration. Injected PAA polymers formed tangled aggregates, which were visible as bright regions in phase contrast optics, and in fluorescence optics when coinjected with fluorescent dextrans (Fig. 1). The polymers were not enclosed in membranes, as evident from the penetration of 70 kDa fluorescent dextrans injected subsequently (not shown). Microtubules were present throughout injected cells, including the region occupied by PAA (Supplementary Material, Fig. S1 *B*), whereas the exclusion of membrane-bound organelles was responsible for the low phase density of PAA aggregates. The injection did not cause any detectable interference to cell migration.

The movement of PAA aggregates, referred to as PAA sensors, reflects forces exerted by the surrounding environment—the sensor should move toward strong pulling forces or weak pushing forces while its shape shows the anisotropy of the forces. PAA sensors injected into the posterior region of steadily migrating cells moved to the anterior lamella region within 10 min at a speed of 84.9 ± 24.7 nm/s ($n = 7$, Fig. 1, *A* and *B*, *arrowhead*), which is ~3.5 times faster than the migration of the nucleus during the same period. In contrast, PAA sensors in the anterior region were never able to move backward to the posterior region. In cells that switched the direction of migration, PAA sensors also relocated from the previous position to the newly defined anterior region within 30 min (Fig. 1, *D*, *E*, and *G*, and Supplementary Material, Movie S1). Although it was difficult to rule out direct forces from motor proteins, the weak interactions of PAA with proteins in general made this unlikely. Dragging by an entangled microtubule network was also possible but unlikely, as the probes seemed able to move to the front from any trailing regions in a steadily migrating cell. A more plausible interpretation was that the movements reflected a

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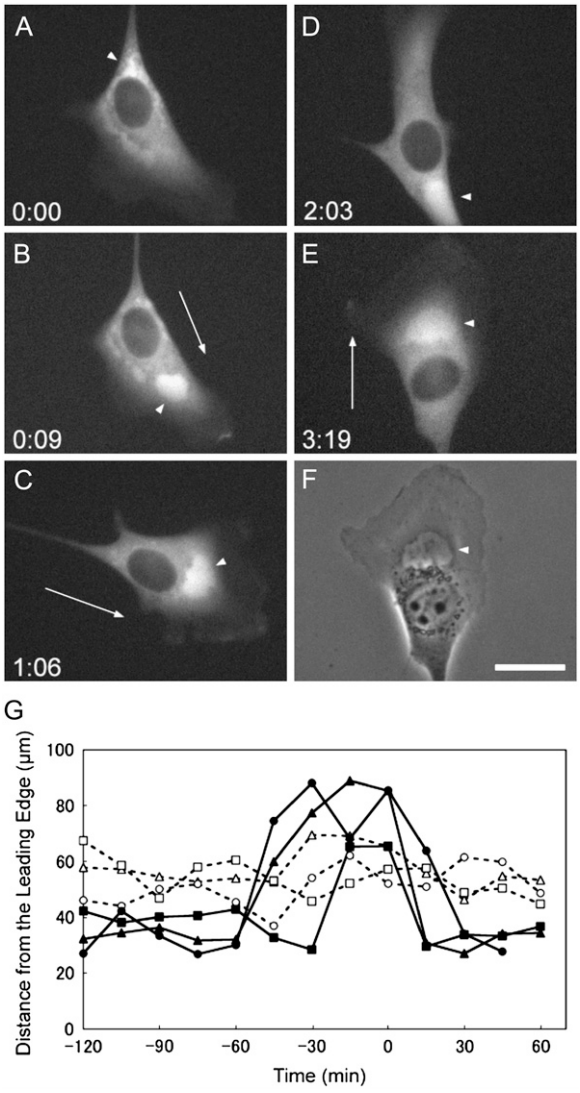

FIGURE 1 Movement of PAA probes in a migrating NIH3T3 cell. Linear PAA, coinjected with fluorescent dextran into the posterior region of a migrating NIH3T3 cell (*A*, *arrowhead*), is visible in phase contrast (*F*, *arrowhead*), and in fluorescence (*A–E*, *arrowhead*) due to the entry of fluorescent dextran and the exclusion of membrane organelles. The probes then move to the anterior lamella region within 10 min (*B*, *arrowhead*). When the cell changes the direction of migration (*D*), PAA probes become temporarily localized to the posterior region, but soon migrate into the new anterior region (*E*, *arrowhead*). Arrows indicate the direction of cell migration. Time after the injection of PAA is shown as h:min. Bar, 20 μm. Plots of the distance of the center of nucleus (*G*, *dotted lines*) and PAA probe (*G*, *solid lines*) from the leading edge in three turning cells show that the nucleus maintains a central location, whereas the PAA probe is initially closer to the leading edge but shows a transient increase in distance during the turn. $T = 0$ indicates the time when the cell resumes directed migration after the turn. Each cell is represented by a distinct symbol.

pressure differential between the posterior and anterior regions.

PAA sensors showed an elongated shape during movement, with the long axis lying parallel to the direction of movement (average aspect ratio 7.6 $\pm$ 2.6 while it overtook the nucleus), suggesting that the probes were either compressed laterally by a gradient of forces or pulled at the front. The relocation of PAA sensors required directional cell migration. PAA sensors remained near the injection site in stationary cells (Fig. 2, *A–C*), and in cells at a medium to high density, where the establishment of migration polarity was hindered by collisions with neighboring cells (Fig. 2, *D–F*). Thus intracellular force gradient is not required for cell migration per se, but for the establishment or maintenance of cell polarity.

To probe the molecular mechanism responsible for the forward movement of PAA sensors, cells injected with PAA were treated with 100 μM blebbistatin, a potent inhibitor of nonmuscle myosin II ATPase ((3); Fig. 3, *A–D*). Blebbistatin-treated cells showed multiple long processes while undergoing random migration at an average speed 60% that of control cells (Fig. 3, *A–D*, *arrows*, and Movie S2). In contrast to control cells, movement of sensors lagged behind that of the nucleus in blebbistatin-treated cells. Inhibition of Rho-dependent kinase by Y-27632 caused a similar response (not shown). As both blebbistatin and Y-27632 are strong inhibitors of traction forces (4), these results suggest that myosin II-dependent cortical contractions, regulated by the Rho-dependent kinase, were responsible for generating the cytoplasmic force gradient.

Previous studies showed that microtubules are required for maintaining cell polarity and migration directionality (5). Coordinated movement of PAA sensors was inhibited within 10 min of treatment with 0.5 μM nocodazole, while PAA sensors scattered and moved toward multiple regions of membrane ruffles (Fig. 3, *E–H*, *arrowheads*, and

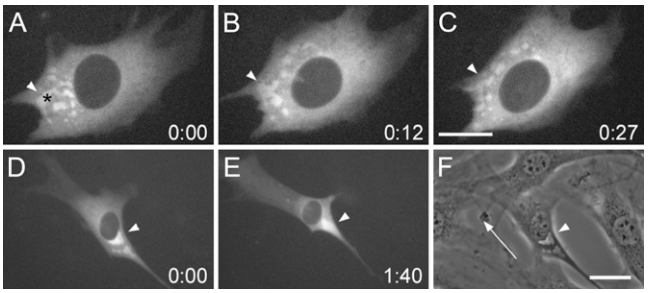

FIGURE 2 Behavior of PAA in nonpolarized cells. PAA injected into a stationary NIH3T3 cell remains as scattered aggregates near the site of injection (*A–C*; *asterisk* indicates the site of injection). When injected into the posterior region of a migrating NIH3T3 cell surrounded by other cells (*D–F*), PAA remains near the injection site (*D* and *E*, *arrowheads*; *F* shows the phase contrast image). Time after the injection of PAA is shown as h:min. Bar, 20 μm.

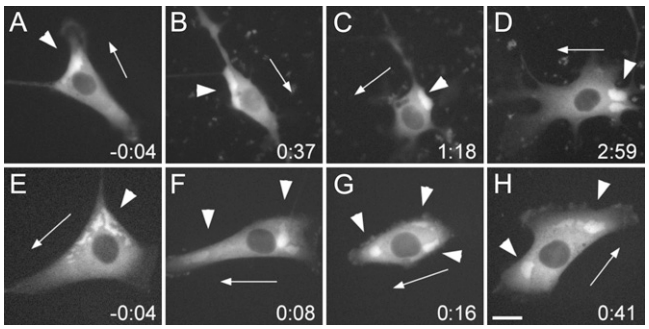

FIGURE 3 Behavior of PAA in drug-treated cells. Migrating NIH3T3 cells injected with PAA (*arrowheads*) are treated with 100 μM blebbistatin (*A–D*) or 0.5 μM nocodazole (*E–H*). The cell treated with blebbistatin shows multiple long projections (*A–D*) and active but random migration, while PAA stayed in the posterior region. In the cell treated with nocodazole, PAA aggregates move toward scattered regions of active membrane ruffles (*E–H*, *arrowheads*). Arrows indicate the direction of the cell migration. Times shown in h:min are relative to the drug treatment. Bar, 20 μm.

Movie S3). These observations suggest that microtubules regulate the localization of membrane ruffles, which may in turn determine the force differential that drives the directional movement of PAA probes.

The forward movement of PAA sensors may be driven by pulling forces at the front, pushing forces at the rear, or a gradient of lateral compressive forces. However, pushing by direct forces from the rear seemed unlikely given the orientation of the probe along the direction of its movement. A plausible mechanism involves a gradient of lateral contractile pressure, generated by strong myosin II-dependent contractions in the posterior cortical shell and progressively weaker contractions toward the front (1). Such pressure gradient may drive the forward transport of intracellular components during cell migration, for example to supply actin subunits for the continuous assembly at the front (6). The mechanism may also be responsible for propelling the transport of large structures such as the nucleus or the microtubule network, which was shown to be dependent on both microtubule motors and acto-myosin interactions (7). Alternatively, PAA sensors may be pulled forward together with the cell body by myosin II-dependent traction forces in the anterior ruffling region (8), as suggested by the movement toward scattered ruffles in cells treated with nocodazole. Other mechanisms such as differential molecular crowding are also worth consideration. Future studies combining experimental manipulations with theoretical soft condensed matter physics should allow more quantitative applications and interpretations of soft polymer aggregates as intracellular force sensors.

MATERIALS AND METHODS

Aqueous solution of 10% (w/v) linear PAA, molecular weight 600,000–1,000,000 (Polysciences, Warrington, PA), was diluted with the injection buffer containing 0.5 mM $MgCl_2$ and 50 mM potassium glutamate (pH 6.5), and mixed with rhodamine isothiocyanate-dextran (molecular weight 70,000 R-9379, Sigma, St Louis, MO) in injection buffer, to obtain a final PAA concentration of 0.5% (w/v) and dextran concentration of 0.5 mg/ml. Blebbistatin (Calbiochem, San Diego, CA) and nocodazole (Sigma) were prepared as 100 mM stocks in dimethylsulfoxide and ethanol, respectively, and diluted into a prewarmed Dulbecco's modified Eagle's medium to a final concentration of 100 μM and 0.5 μM, respectively, before application to cells. Images were collected with a Zeiss Axiovert 100 microscope (Carl Zeiss, Thornwood, NY), equipped with a 40×/NA 0.75 Neofluar objective lens and a cooled CCD camera (NTE/CCD-512-EBFT; Roper Scientific, Trenton, NJ) using custom software.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

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