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Investigation of the Mechanism of Retraction of the Cell Margin and Rearward Flow of Nodules during Mitotic Cell Rounding

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We have studied two types of cell motility directed toward the cell center: retraction of the cell margin and rearward flow of small cytoplasmic nodules during mitotic cell rounding in Potoroo tridactylis kidney (PtK2) cells by time-lapse video microscopy, drug treatments, and photoactivation of fluorescence. Nodules flow rearward on thin, actin-rich fibers (retraction fibers) exposed as the cell margin retracts. Retraction of the cell margin and rearward flow of nodules require intact actin filaments, but are insensitive to an inhibitor of myosin function (butanedione monoxime). Using photoactivation of fluorescence marking, we have determined that actin filaments in the majority of retraction fibers remain stationary while the cell margin retracts and nodules flow rearward. The pointed ends of retraction fiber actin filaments face the cell center. We argue that nodule motility is driven by a novel actin-based force that perhaps also partially contributes to retraction of the cell margin during cell rounding at mitosis.

INTRODUCTION

Cell shape change plays an important role in many areas of biology. Models for cell morphogenesis describe shape changes that occur as a result of motility directed away from the cell center (e.g., protrusion of cell margins such as lamellipodia and filopodia, cell spreading after mitosis). However, equally important is shape change resulting from motility directed toward the cell center (e.g., retraction of the cell margin). Retraction from the substrate occurs during cell rounding at mitosis, at apoptosis, and during oncogenic transformation. The cell margin also retracts at the rear of migrating cells. To facilitate comparison between different motile systems, we define movement directed away from the cell center as "forward" (also termed outward or anterograde in the literature) and movement directed toward the cell center as "rearward" (also termed inward, backward, or retrograde in the literature).

Very little is known about the mechanism of retraction of the cell margin. In this article, we focus on retraction of the cell margin during cell rounding at mitosis. A round cell shape at mitosis probably plays a role in setting the stage for subsequent cytokinesis. Surprisingly, there is no complete description of retraction of the cell margin in rounding cells and the mechanism of this motility during mitotic cell rounding has been little studied.

Mitotic cell rounding is a reproducible and easily studied process in flat tissue culture cells (Harris, 1973; Wetzel *et al.*, 1978), e.g., PtK2[1] cells (Sanger *et al.*, 1984; Mitchison, 1992). During mitotic cell rounding in PtK2 cells and other highly adhesive cells, two types of motility occur toward the cell center. The first is retraction of the cell margin. As the cell margin retracts a network of long, thin, actin-rich fibers (retraction fibers) attached to the substrate at their distal end are exposed, connected at right angles to the cell margin at their proximal end. Revealed on these retraction fibers is the second type of motility directed toward

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[1] Abbreviations used: BDM, butanedione monoxime; PtK2, Potoroo tridactylis kidney.

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the cell center; rearward flow of small, discrete cytoplasmic nodules (Mitchison, 1992). Although it is not known whether nodule motility contributes to the cell rounding process per se, for several reasons in this article we have studied both retraction of the cell margin and rearward nodule flow in mitotic PtK2 cells. We suspect that rearward nodule flow reflects a mechanism that is at least partially common to retraction of the cell margin in rounding mitotic cells. Thus, studying rearward nodule flow, which is a relatively simple type of motility, may provide insight into the more complex cell rounding process at mitosis. Also, rearward nodule flow and retraction of mitotic cell margins resemble features of other retractile cell types and rearward types of cell motility. Thus, an investigation of both rearward nodule flow and retraction of mitotic cell margins may yield information on retractile and rearward motile mechanisms in general. To characterize the system, we first describe the detailed dynamic morphological changes that occur during mitotic cell rounding. We then address potential mechanisms for retraction of the cell margin and rearward nodule flow by analyzing the effects of drugs that affect cytoskeletal proteins, since these proteins are implicated in many different types of cell motility. Finally, guided by the results of our drug studies, we report the dynamic behavior of actin filaments in retraction fibers in live mitotic cells determined by photoactivation of fluorescence. Our studies provide insight into the mechanism of retraction of the cell margin and rearward nodule flow during mitotic cell rounding in PtK2 cells.

MATERIALS AND METHODS

Cell Culture

PtK2 cells, an epithelial cell line, were obtained from the American Type Culture Collection (Rockville, MD) and grown in DME-H16 medium with 10% fetal calf serum, streptomycin, and penicillin at 37°C in 5% CO_2. One to 3 days before an experiment, they were plated onto glass coverslips and transferred on the day of observation to an aluminum chamber. Cells were observed at 34–36°C in bicarbonate- and phenol red-free DME with 10% bovine calf serum, 20 mM HEPES (pH 7.4), streptomycin, and penicillin. This was covered with a thin layer of silicone oil to prevent evaporation.

Video Microscopy for Drug Studies

Time-lapse phase-contrast video recording of unperturbed and drug-treated mitotic cells was done with a charge-coupled device video-rate camera (Hamamatsu, Bridgewater, NJ). A 40× Neofluar 0.75 NA objective with a 1× eyepiece was used. Cytochalasin D (Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 10 μg/ml and butanedione monoxime (BDM, Sigma Chemical Co.) to a final concentration of 10–20 mM to cells under observation during early, mid, or late rounding for 10 to 30 min. Drugs were washed out with three to four changes in phosphate-buffered saline and then two to three changes in media. BDM is a low-affinity inhibitor of myosin, but seems specific for this protein. In vitro BDM inhibits purified rabbit muscle myosin II (Higuchi and Takemori, 1989), purified human nonmuscle myosin II and pig myosin V; and a mixture of *Drosophila* myosins I, II, and VI (Cramer and Mitchison, 1995). In the same study, in vivo BDM disrupts motility based on myosin II (cytokinesis) in Ptk2 cells and myosin 1C (vacuole contraction) in *Acanthamoeba*. Also, in PtK2 cells, BDM disrupts the organization of myosin II in cell-staining studies. In a different study in *Aplysia* neurons, BDM inhibits rearward flow in lamellipodia (Lin *et al.*, 1996). It does not interfere with kinesin ATPase activity nor actin filament assembly.

Microinjection and Photoactivation

Microinjection of caged resorufin actin into PtK2 cells, photoactivation of fluorescence, and collection of paired phase-contrast and epifluorescence images with a silicon-intensified target-tube camera were performed as described (Mitchison and Cramer, 1993).

Preparation of Images

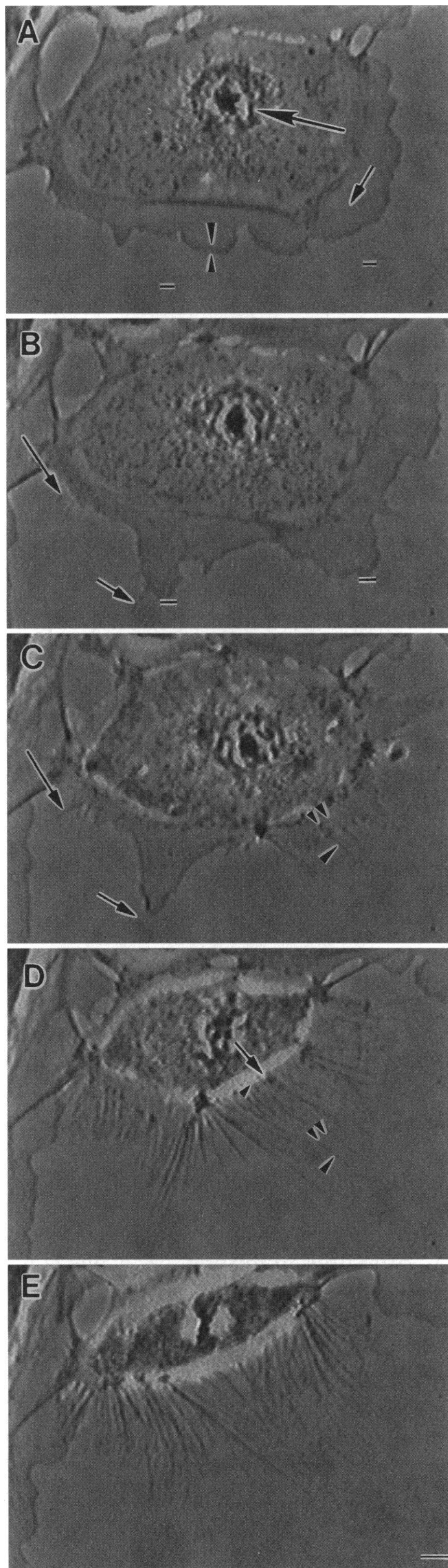
All video images were imported into Adobe photoshop (Adobe Systems Inc., Mountain View, CA), digitally processed, and printed on a Phaser 440 printer (Tektronix Inc., Wilsonville, OR).

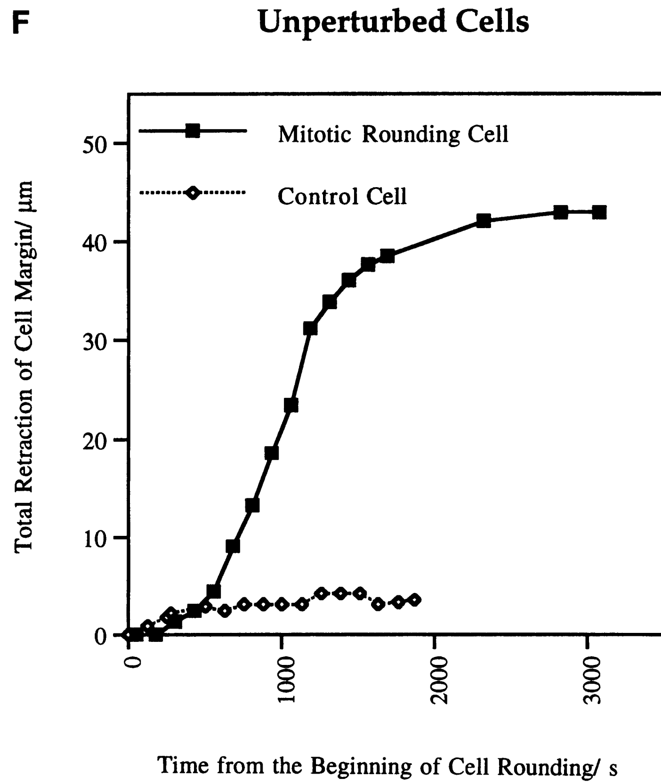
RESULTS

Characterization of Mitotic Cell Rounding in PtK2 Cells

Mitotic cell rounding in PtK2 cells starts during prophase as defined by the morphology of chromosomes in live cells (Figure 1). Early in prophase, before the onset of cell rounding, the cell margin is a narrow lamellipodium (Figure 1A, between the arrowheads), typical of interphase PtK2 cells. Sandwiched between

Figure 1 (on facing page). Retraction of the cell margin during mitotic cell rounding. Time-lapse video microscopy of a mitotic PtK2 cell before (A and B) and during cell rounding (C–E). (A) 0 s. The cell is in early prophase with condensing chromosomes (long arrow) and has a lamella (short arrow) and a lamellipodium (between the arrowheads). (B) 1869 s (13.2 min). Filopodia (long and short arrow) and lamellipodia (compare fixed horizontal lines in A and B) protrude. (C) 3234 s (53.9 min), early stage of rounding. The lamella (points of the double arrowhead) retracts exposing retraction fibers (e.g., arrowhead). Filopodia either remain attached to the substrate (long arrow) or have retracted (short arrow in C indicates the position of the filopodium in B). (D) 4284 s (71.4 min), middle stage of rounding. The cell margin is the edge of the bulk cell body and it retracts to the arrow from its position in C (backs of the double arrowhead) with further exposure of retraction fibers (between the single arrowheads). The phase-bright rings in D and E (D, arrow) are due to the roundness of the cell. (E) 5334 s (88.9 min), continuing middle and late stage of rounding. The cell margin retracts until the end of rounding. (F) Total distance that a representative cell margin (■) retracts from the beginning of cell rounding (set at 0 s, which was 3200 s (53.3 min) from the beginning of filming). The stages of rounding are early (lag phase); middle (linear portion of the curve); late (shoulder of the curve); and end (plateau at the top of the curve). The control margin (◇) is a lamellipodium of an interphase PtK2 cell. (G) The average rate of retraction is 0.63 ± 0.05 μm/min (early and in some cells beginning of middle stage), 2.26 ± 0.21 μm/min (middle stage), 0.67 ± 0.08 μm/min (late stage), and overall is 1.30 ± 0.11 μm/min, n = 140 edges (19 cells). ± and the error bars are SEMs. Bar, 10 μm.





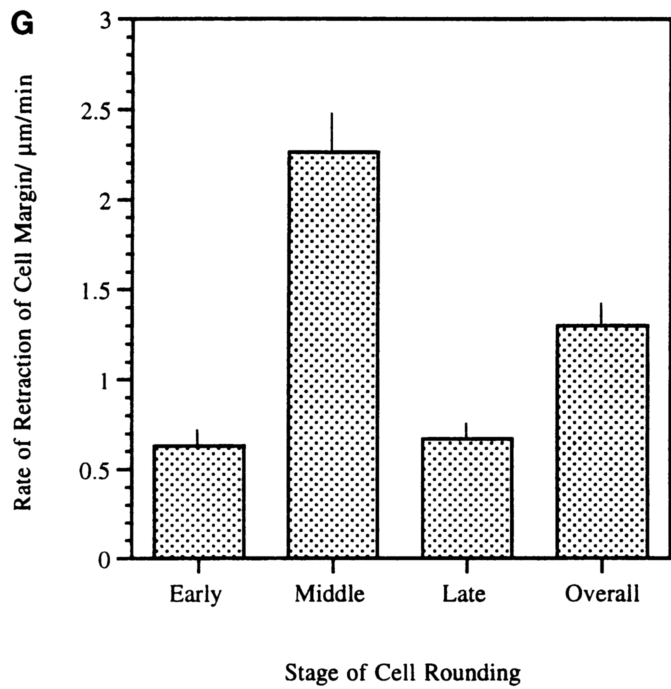


Figure 1.

the lamellipodium and the bulk cell body is a wider band of distinct morphology called the lamella (Figure 1A, short arrow). Before the cell rounds, we observe an increase in the frequency at which lamellipodia (Figure 1B, horizontal lines) and filopodia (Figure 1B, long and short arrows) protrude and retract. Once the lamellipodium has retracted for the last time, the lamella becomes the cell margin. We define the beginning of cell rounding when lamella retraction starts (Figure 1C, points of the double arrowhead). Continuing lamella retraction constitutes the early stage of rounding. As the lamella retracts retraction fibers are exposed (Figure 1C, single arrowhead). Filopodia that protrude before cell rounding either retract (Figure 1, compare B and C, short arrow), or stably attach to the substrate and become indistinguishable from retraction fibers in morphology and dynamic behavior (Figure 1, compare B and C, long arrow). The number of filopodia observed to protrude and stably attach to substrate before rounding is small compared with the number of retraction fibers exposed during rounding. Once the lamella has completely retracted, the edge of the bulk cell body becomes the cell margin. This retracts significantly (Figure 1D, arrow, middle stage of rounding) with a concomitant increase in the length of retraction fibers (Figure 1D, between the single arrowheads). Retraction of the cell margin continues until the cell is completely rounded (Figure 1E, late stage of rounding). Retraction of the cell margin during unperturbed mitotic cell rounding is a continuous process (Figure 1F), but the rate varies at different stages of cell rounding (Figure 1G). The overall time it takes individual cells to completely round from the beginning of lamella retraction varies between 25 and 70 min (1500–4200 s) averaging about 40 min at 34–36°C. Time spent in mid-rounding is roughly 15–30 min. The total distance the cell margin retracts ranges from 20 to 55 μm. For a description of the subsequent respreading process after mitosis, see Cramer and Mitchison (1993, 1995).

As retraction of the cell margin exposes retraction fibers during early, mid, and late rounding, we observe nodules flowing rearward on them. Nodule flow also continues after there is no more retraction of the cell margin (Mitchison, 1992). An example of a nodule on a retraction fiber is shown in Figure 2A. Here, we show that the rate of nodule flow increases with each stage of cell rounding (Figure 2B).

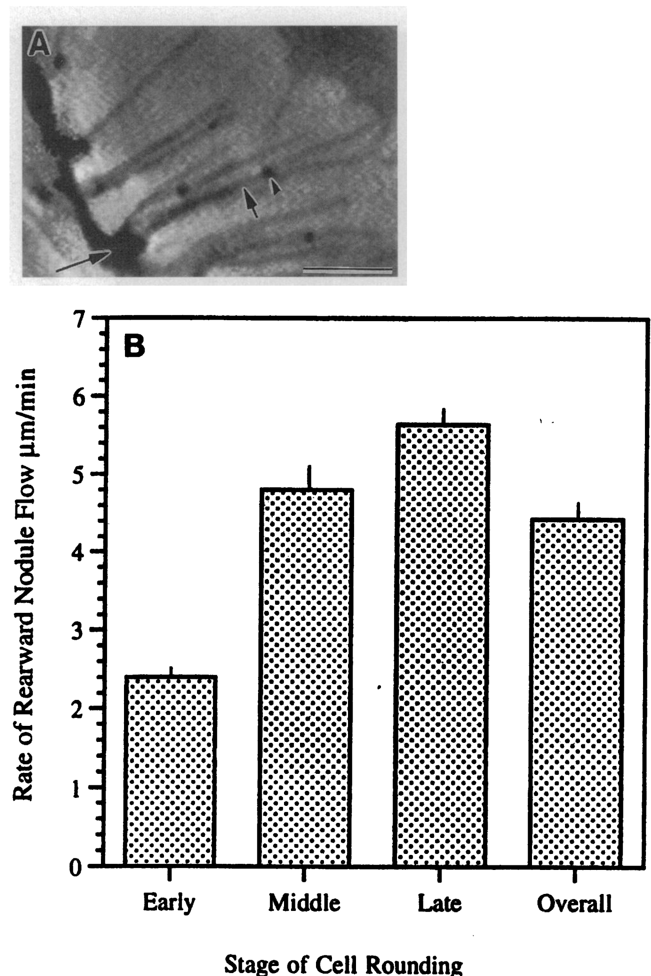
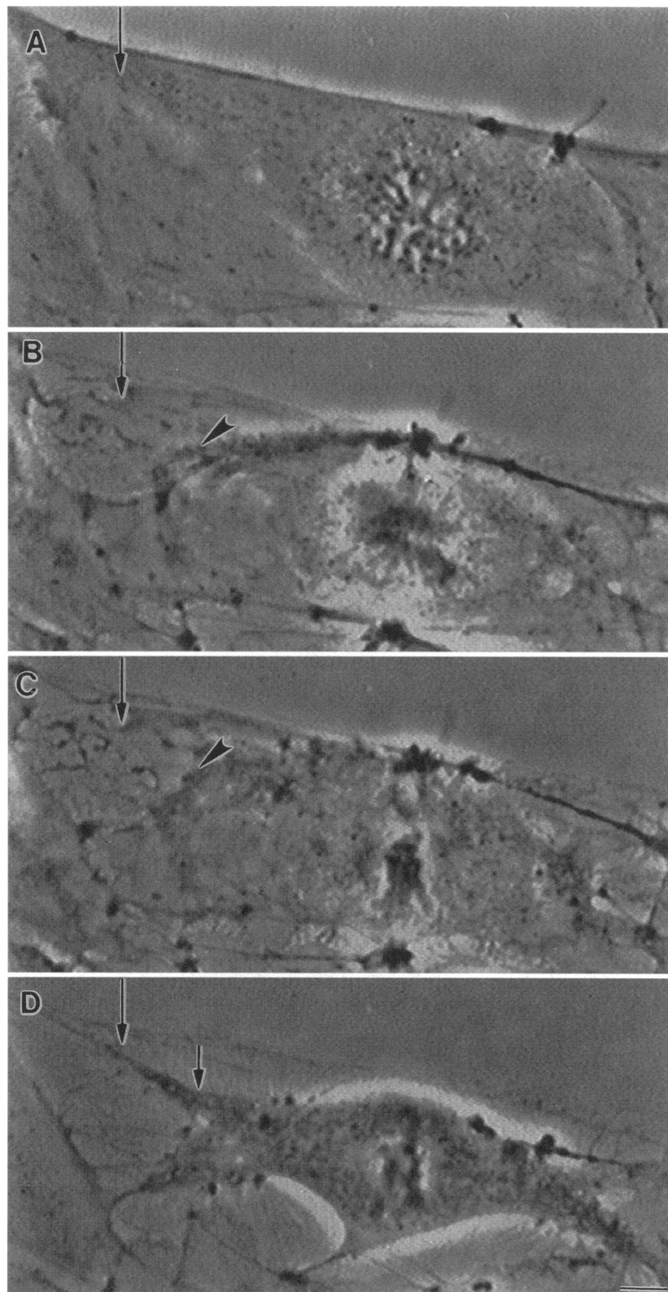


Figure 2. Rearward nodule flow during mitotic cell rounding. (A) An enlarged phase-contrast view of a portion of a cell margin in a mitotic PtK2 cell (long arrow). Nodules appear as small phase-dense particles (e.g., arrowhead) on retraction fibers (e.g., short arrow). (B) The average rate of rearward nodule flow is 2.40 ± 0.09 μm/min (early stage of rounding), 4.80 ± 0.33 μm/min (middle stage of rounding), 5.64 ± 0.16 μm/min (late stage of rounding), and overall is 4.43 ± 0.13 μm/min, n = 271 (18 cells). Bar, 10 μm.

Cytochalasin D Disrupts and Inhibits Retraction of the Cell Margin during Mitotic Cell Rounding

We next investigated potential mechanisms for retraction of the cell margin and rearward nodule flow during mitotic cell rounding. Other types of cell motility directed toward the cell center in other systems are known to be dependent on actin filaments (Sheetz *et al*, 1989; Forscher and Smith, 1990). Also, we knew from a previous study (which we confirm below) that rearward nodule flow in mitotic PtK2 cells is inhibited by cytochalasin D (Mitchison, 1992). A role for actin filaments in retraction of the cell margin during mitotic cell rounding has not previously been reported.

The effect of cytochalasin D treatment on retraction of the cell margin during early or mid-rounding is dramatic. It shears the cell into distinct dorsal (upper) and ventral (lower) regions, each with its own distinct margin. Within 30–60 s of treatment it causes rapid retraction of the dorsal region that includes most of the cell cytoplasm (Figure 3B, arrowhead). This is complete in 5–10 min and on average is about seven times faster than the overall average rate of unper-



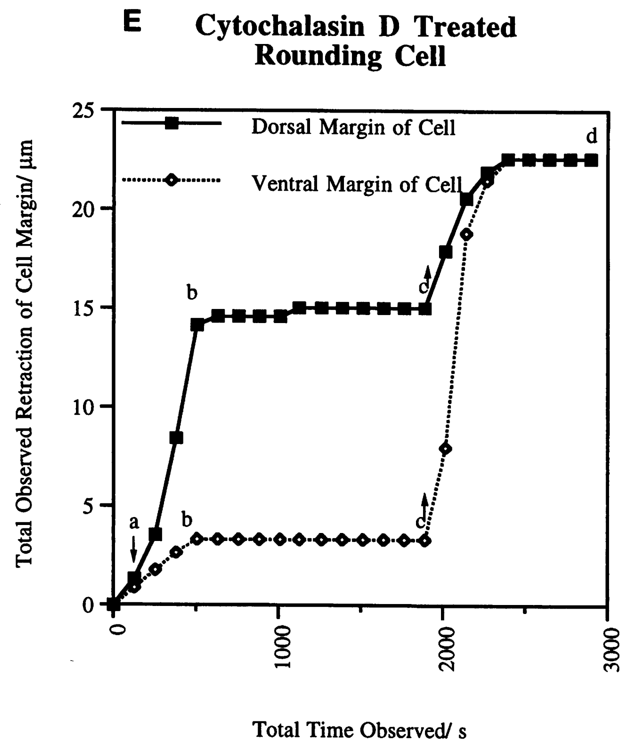


Figure 3. Cytochalasin D disrupts and inhibits retraction of the cell margin during mitotic cell rounding. Long arrows in (A–D) mark a fixed position in A, and arrowheads in B and C mark a fixed position in B. (A) 126 s (2.1 min). A mitotic cell at the end of the early stage of cell rounding (most of the lamella has retracted) just as cytochalasin D is added to the media. At this stage in this cell, the exposed retraction fibers are short (and in this image barely discernible). (B) 483 s (8.1 min). During the first 357 s (6.0 min) of cytochalasin D treatment, the dorsal (upper) region of the cell rapidly retracts from the arrow to the arrowhead, but the ventral (lower) cell region remains spread (between the arrow and arrowhead). (C) 1827 s (30.5 min). Cytochalasin D treatment inhibits retraction of both cell margins of the dorsal (arrowhead) and ventral (arrow) cell regions. (D) 2919 s (48.7 min). Retraction resumes in washout (washout was at 1848 s [30.8 min]), e.g., for the ventral margin of the cell from the long arrow to the short arrow. Resumption of retraction of the dorsal margin of the cell is not conveyed by still images. (E) Total retraction of the dorsal (■) and ventral (◇) margins of the cell in A–D. Cytochalasin D was added at 126 s (2.1 min, downward arrow) and washed out at 1848 s (30.8 min, upward arrow). Lines before the downward arrow represent the intact cell edge. The letters (a–d) refer to the appearance of the cell in A–D. Bar, 10 µm.

turbed cell retraction, but in individual cells can be up to 40 times faster. In time-lapse the dorsal surface appears to snap rearward as if part of a taut elastic band was cut. Retraction of the dorsal cell margin is inhibited with continued cytochalasin D treatment (Figure 3, compare B and C, arrowhead). The ventral region remains spread during dorsal retraction in cytochalasin D (Figure 3B, between the arrow and arrowhead). Cytochalasin D simply inhibits retraction of the ventral cell margin after 1–6 min of total treatment (Figure 3, compare B and C, between the arrow and arrowhead). Inhibition of retraction is easily seen in the distance versus time plot (Figure 3E) as compared with unperturbed cells (Figure 1F). Retraction of both margins resumes after 1–2 min of washout at expected unperturbed rates to form a normal rounded cell (Figure 3D). Often at the onset of washout the ventral cell margin appears to break up into nodules, and rearward flow of these nodules rather than retraction of an intact margin contributes to forming the normal rounded cell. Sometimes no further retraction of the dorsal cell region was observed in washout if the

initial rapid retraction in cytochalasin D was fast enough to prematurely completely round this region of the cell body. Retraction fibers do not contain microtubules, and nocodozole neither affects retraction (Sanger *et al.*, 1984) nor nodule flow (our unpublished observations) during mitotic cell rounding.

Retraction of the Cell Margin and Rearward Nodule Flow Is Insensitive to BDM during Mitotic Cell Rounding

Actin filaments are required for retraction of the cell margin and rearward nodule flow during mitotic cell rounding, but what is the likely source of force generation? Some types of motility directed toward the cell center have been shown to be driven by myosin II (Pasternak *et al*, 1989; Fukui *et al.*, 1990). Others have been argued to be powered by different myosins (Grebecki, 1994; Mitchison and Cramer, 1996). We tested these ideas for retraction of the cell margin and rearward nodule flow in our system by treating mitotic PtK2 cells with a myosin inhibitor, BDM. We have previously extensively characterized and described the use of this drug. It specifically inhibits the ATPase activity of at least three different myosins, including myosin II from four different species in vitro, and disrupts myosin-based motility in two other species in vivo (see MATERIALS AND METHODS). Both retraction of the cell margin and rearward nodule flow are unaffected by this drug (shown for a representative cell margin and nodules in Figure 4). Under the same BDM conditions, respreading after mitosis in PtK2 cells is completely blocked (Cramer and Mitchison, 1995). Quantitation of the average rates of retraction and rearward nodule flow in cytochalasin D and BDM, respectively, are shown in Table 1.

Actin Filaments Are Stationary in Retraction Fibers during Mitotic Cell Rounding

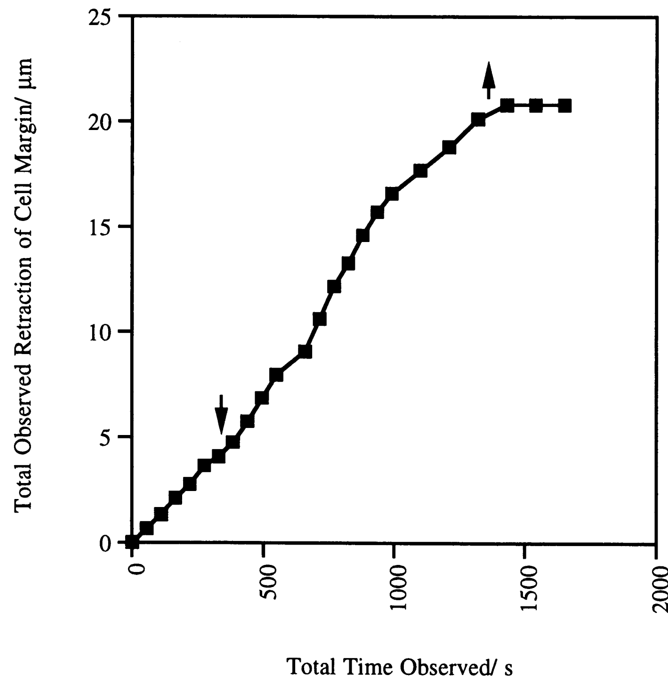
It has also been suggested for some types of rearward motility associated with the dorsal cell surface that the motility is driven by coupling to the rearward movement of underlying (i.e., more ventral) actin filaments (Sheetz *et al.*, 1989; Lin and Forscher, 1995). In mitotic cells, nodules and the bulk of the cell itself are typically more dorsal than retraction fibers which being the cells attachment point to substrate are more ventral. Since retraction fibers contain actin filaments (Mitchison, 1992; Cramer and Mitchison 1993, 1995), it is formally possible that these filaments move rearward and that nodules flow rearward on retraction fibers because they are passively attached to this moving underlying actin. The same is also possible for the cell margin itself. This is because in time lapse the cell margin appears to move over retraction fibers, i.e., as the margin retracts, more and more of the final length of a retraction fiber is revealed (Figure 1). We therefore wished to determine whether actin filaments in retraction fibers were moving rearward in mitotic cells. We have previously determined that actin filaments in retraction fibers are stationary during postmitotic cell spreading (Cramer and Mitchison, 1993). Here, we test the behavior of these filaments in mitotic cells by photoactivation of fluorescence. Retraction fibers were marked either during retraction of the cell margin when rearward nodule flow is also occurring (7 cells) or after retraction of the cell margin when nodules are still flowing (19 cells). In these 26 mitotic cells, 98% (42/43) of marked and observed retraction fibers contain stationary actin filaments with respect to the substrate. The other 1 of 43 moves forward. Stationary actin filaments in retraction fibers are shown for a representative mitotic cell (Figure 5, C–H) with a retracting cell margin (Figure 5, A and B) and a rearward flowing nodule (Figure 5, E–G).

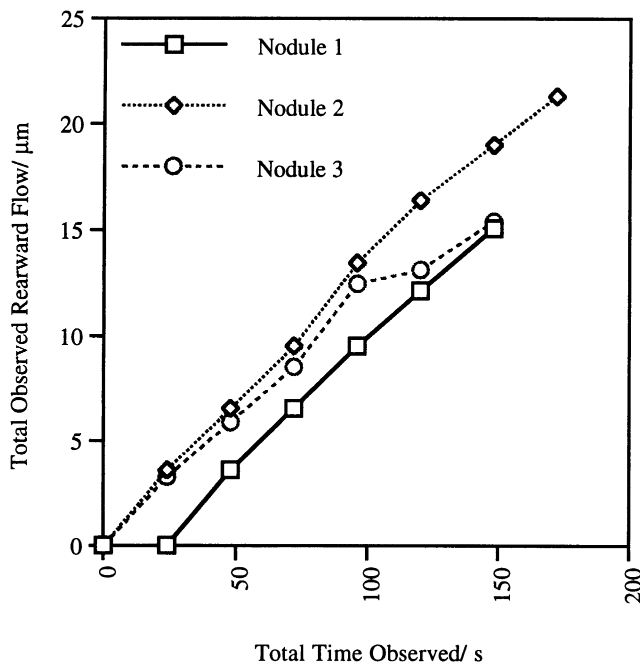
Nodules and retracting margins themselves also contain actin filaments (Mitchison, 1992), therefore, we expected to be able to also mark actin in nodules and retracting margins and that this actin would move during the course of our photoactivation experiments. With respect to nodules, we observed 22 actin marks moving rearward; 21 of these marks were clearly nodules. The other mark was a more inhomogenous phase density which we believe was an out-of-focus nodule. When 7 of these nodules and the phase-dense inhomogeneity were marked (8/22 = 36%), we also detected fluorescent actin filaments in the retraction fiber containing the nodule/inhomogeneity. As expected, the actin in the nodule/inhomogeneity moves rearward and the actin in the retraction fiber remains stationary, resulting in a separation in the original mark (Figure 5I). We were unable to mark many retracting cell margins. This is because it is not easy to predict after microinjecting prophase cells with caged fluorescent actin when the cell will start to round (unlike cell spreading after mitosis, for example, which always starts at the end of cytokinesis). Also, the most active stage of cell retraction (mid-rounding) only lasts for 15 to 30 min. The retracting cell margins that we did mark (in three mitotic cells) behave like nodules in that there is a separation in the original mark as the margin retracts; actin in the cell margin moves rearward with the margin and underlying actin in the newly revealed retraction fibers remains stationary.

DISCUSSION

Rearward Nodule Flow Reflects a Common Motile Mechanism

Rearward nodule flow is always coincident with retraction of the cell margin, occurring continuously through each stage of cell rounding (Figure 2). We





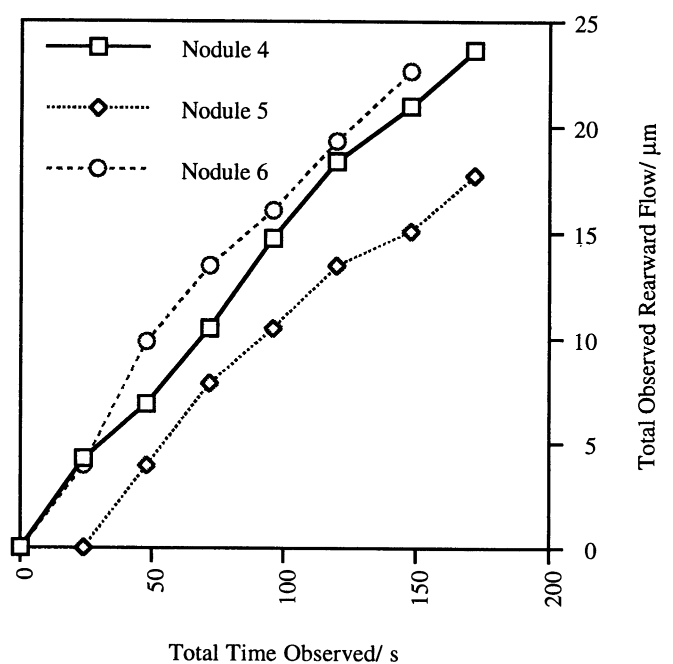


Figure 4. BDM does not inhibit retraction of the cell margin and rearward nodule flow during mitotic cell rounding. Representative retraction of a cell margin starting at the middle stage of rounding (A) and for representative nodules flowing rearward on different retraction fibers of the same cell (B and C). (A) Retraction continues normally after BDM is added at 396 s (6.6 min, downward arrow). BDM was washed out at 1375 s (22.9 min) toward the end of cell rounding (upward arrow) and the distance the margin then retracts plateaus as expected. (B and C) Rearward nodule flow was measured before (B) and during a minimum BDM treatment of 786 s (13.1 min). Each curve represents a different nodule.

Table 1. Quantitation of cytochalasin D and BDM data for retraction of the cell margin and rearward nodule flow during mitotic cell rounding

Treatment	Retraction of Cell Margin: Rate (μm/min) ± SEM	n	Rearward Nodule Flow: Rate (μm/min) ± SEM	n
Cytochalasin D				
Before	0.94 ± 0.22	15	3.30 ± 0.23	40
During (shearing)	7.65 ± 0.65	35	not applicable	
During (inhibition)	0.01 ± 0.00	32	0	41
Washout	1.83 ± 0.18	34	4.44 ± 0.19	67
BDM				
Before	0.96 ± 0.08	59	4.97 ± 0.25	50
During (no effect)	1.12 ± 0.09	62	5.40 ± 0.24	55
Washout	0.70 ± 0.08	28	5.15 ± 0.28	49

Average rates of motility are shown for 10 to 17 cells. We typically added drugs to cells during early or mid-rounding. Rates of retraction of the cell margin and rearward nodule flow in untreated cells depend on the stage of rounding (Figures 1G and 2B); therefore, we expected the observed small differences in average motile rates of the cell population before drug treatment versus washout.

think this reflects a motile mechanism that is common to both nodules and the cell margin. Other motile mechanisms are also thought to be shared among various types of cell motility (Sanger and Sanger, 1992; Theriot and Mitchison, 1992a; Cramer *et al.*, 1994). In mitotic rounding cells a shared mechanism is spatially possible because nodules and the cell margin are in close proximity to each other. More compelling is the observation that retraction of the margin of the ventral cell region, inhibited in cytochalasin D, often occurs by apparent breaking up of the margin into rearward flowing nodules during washout (see the legend to Figure 3). Nodule flow might be more generally indicative of a specific retractile or rearward movement. For example, we have seen what appear to be nodules on retraction fibers at the rear end of migrating cells (e.g., Figure 2 in Chen, 1981; Figure 49 in Ramsey, 1972) and have observed that they flow rearward (toward the cell center; Cramer and Mitchison, unpublished data).

A Mechanism for Rearward Nodule Flow during Mitotic Cell Rounding

In mitotic cells nodule flow on retraction fibers requires intact actin filaments (Mitchison, 1992; Table 1). Proposed motile mechanisms for actin-dependent motility are based on passive attachment of the moving structure to moving actin or on myosin forces (Sheetz, 1993; Grebecki, 1994; Mitchison and Cramer, 1996). Our data exclude the possibility that nodules are moving by passive attachment to underlying moving actin filaments in retraction fibers, because actin filaments in retraction fibers do not move when nodule flow is occurring on them (Figure 5, E–I). Although nodules themselves also contain actin which moves with the nodule (Figure 5I), the same question remains, how does the nodule/actin in the nodule move on retraction fibers which are comprised of stationary actin filaments? Is rearward nodule flow driven by myosin activity? Rearward nodule flow during mitotic cell rounding is insensitive to the myosin inhibitor BDM (Figure 4, B and C, and Table 1). Since BDM inhibits a number of myosins and myosin-based motility in different species, it is likely to be a general myosin inhibitor (for details, see MATERIALS AND METHODS). In particular, BDM disrupts the organization of myosin II in PtK2 cells, the same cells studied here. These data make it very unlikely that myosin II- and V-based forces drive rearward nodule flow and do not favor those based on myosin I. That myosin II-based mechanisms are unlikely is consistent with the current thinking that myosin II may be inhibited in mitosis (Satterwhite *et al.*, 1992; Yamakita *et al.*, 1994). Together these data lead us to conclude that rearward nodule flow is a novel type of actin-based motility. How might it be driven? The pointed ends of retraction fiber actin filaments mostly face the direction of rearward nodule flow (Cramer and Mitchison, 1995). In light of this data, one simple explanation for a force that drives rearward nodule flow during mitotic cell rounding is activity of a novel pointed end-directed actin-based motor (Figure 6). No such motors have been identified biochemically, but it is not clear that they have been actively sought.

How Does the Cell Margin Retract during Mitotic Cell Rounding?

We think that more than one process contributes to retraction of the cell margin during mitotic cell rounding because of the complexity of the cytochalasin D

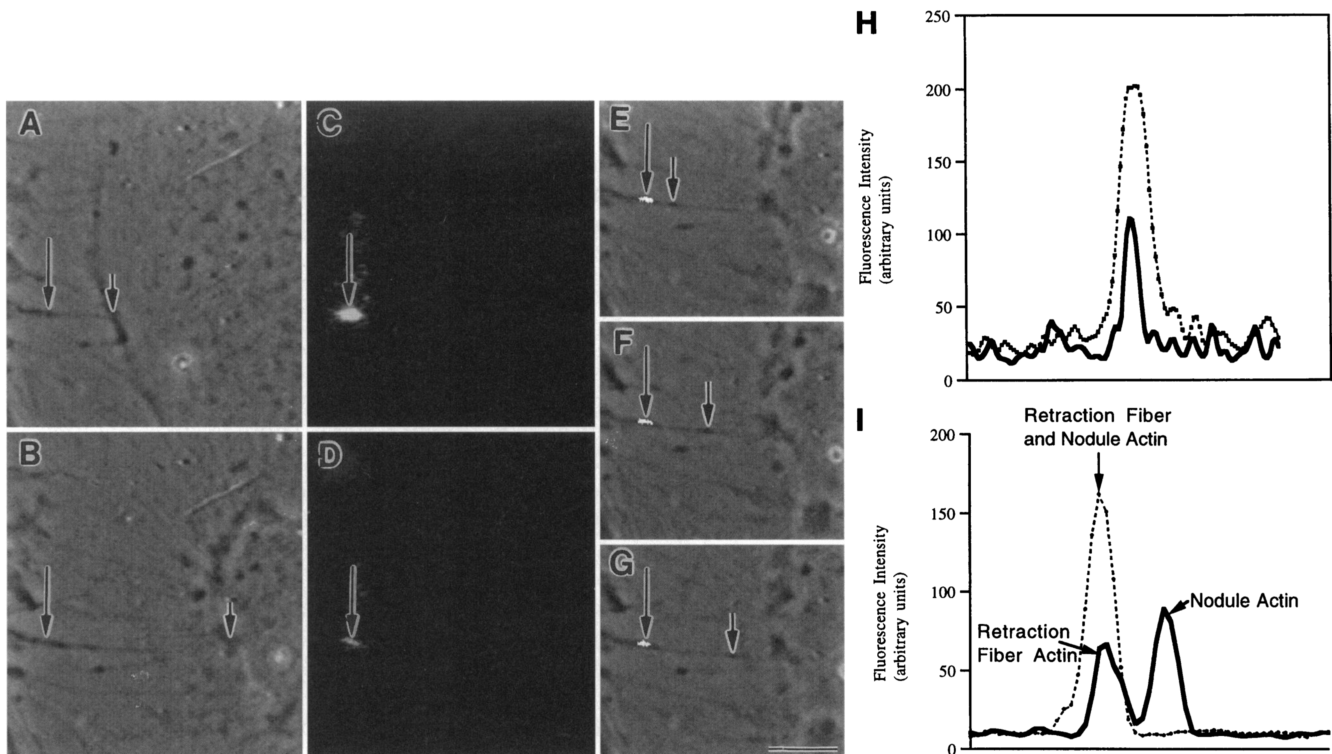
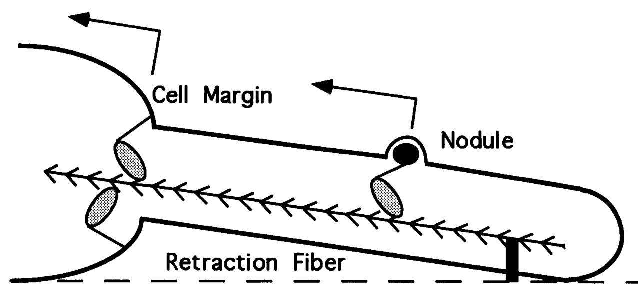


Figure 5. Actin filament dynamics in retraction fibers (A–I) and nodules (I) marked by photoactivation of fluorescence during mitotic cell rounding. Paired (A–D) or superimposed (E–G) phase-contrast and epifluorescence images are shown. Long arrows in A–D and E–G point to fixed positions in A and E, respectively. (A and B) The cell margin retracts from the short arrow in A to the short arrow in B at an average of 0.85 μm/min over 1140 s (19.0 min). (C–G) The actin filaments in the marked retraction fiber are stationary (long arrow) at 2 s after photoactivation (C) and 1140 s (19.0 min) later (D), as the cell margin retracts, and during the 124 s (2.1 min) that an observed nodule (E–G, short arrow) flows rearward at 3.65 μm/min. (H) Fluorescence intensity profiles of the marked retraction fiber shown in C and D at 2 s after photoactivation (dashed line) and 1142 s (19.2 min) later (solid line). (I) Marked retraction fiber and nodule actin (dashed line, 0 s) separates 325 s (5.4 min) later (solid line) as the nodule flows rearward on the retraction fiber. The average half-life of actin filaments in retraction fibers is 800 s (13.3 min ± 1.7 min), n = 7 cells. Bar, 10 μm.

effects (Figure 3). At least one contributing process is actin-based, because retraction of the ventral region of the cell is inhibited in the presence of this drug. How might this actin-based process be driven? As argued above for nodules, our data on retraction do not support mechanisms based on underlying moving actin filaments in retraction fibers nor on myosin activity (Figures 4A and 5, A–D; Table 1). Similarly, this leads us to the conclusion that retraction of the cell margin during mitotic cell rounding includes a novel type of actin-based force. We suspect that retraction of the cell margin and rearward nodule flow share a common motile mechanism, leading us to propose one source of force generation for retraction of the cell margin and rearward nodule flow during mitotic cell rounding (Figure 6). For retraction this model requires that retraction fiber actin in mitotic rounding PtK2 cells should not be restricted to the length of the retraction fiber, but continue as a bundle of actin within the cell margin (as drawn in Figure 6). We have observed this in the same cells during postmitotic cell spreading (Cramer and Mitchison, 1993, 1995) and have confirmed it for mitotic PtK2 cells (Cramer and Mitchison, unpublished data).

Is retraction of the cell margin during mitotic cell rounding entirely an actin-dependent process? Sudden rapid retraction of the dorsal region of the mitotic cell in cytochalasin D during early or mid-rounding is difficult to interpret (Figure 3). In interphase in other types of motile cells, e.g., motile fibroblasts and glial cells, cytochalasin also causes relatively rapid retraction (Spooner *et al.*, 1971; Croop and Holtzer, 1975). Sudden retraction is the expected phenotype of an adherent object under tension that rapidly loses adhesion. This explanation is consistent with the observation that sudden retraction is minimal later in mitotic rounding when cells are already largely retracted (i.e., less adherent) and thus expected to be under less tension. Actin filaments are an important part of adhesion complexes (Yamada and Miyamoto, 1995). The rapid effect of cytochalasin D on rounding cells may be due to rapid loss of actin-dependent adhesion. We



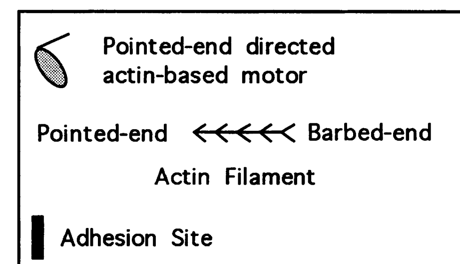


Figure 6. Potential source of force generation for retraction of the cell margin and rearward nodule flow during mitotic cell rounding. Our data do not support moving actin filaments in retraction fibers and a number of myosins as candidates for driving actin-based retraction of the cell margin and rearward nodule flow. We know that the pointed ends of actin filaments in retraction fibers face the direction of retraction of the cell margin and rearward nodule flow. We suggest that a putative pointed end-directed actin-based motor drives nodules rearward. Such a motor may also contribute to retraction of the cell margin.

do not know whether more controlled loss of actin-dependent adhesion plays an active role in driving normal mitotic cell retraction. Mitotic retraction is generally slower than nodule motility (compare Figures 1G and 2B), perhaps because retractile force normally has to work against an opposing adhesive force.

Relevance of Retraction of the Cell Margin and Rearward Nodule Flow for other Types of Cell Motility Directed Toward the Cell Center

Although the source of force generation is known for a few types of motility directed toward the cell center (Pasternak *et al.*, 1989; Fukui *et al.*, 1990), others are either poorly understood, e.g., rounding of cells during apoptosis and oncogenic transformation, or controversial, e.g.., rearward flow in lamellipodia. In general, models for retraction of the cell margin in other rounding cell types have focused on the role of adhesion and myosin II-driven motility in permeabilized cells (Sims *et al.*, 1992; Crowley and Horwitz, 1995). Interpreting results of experiments to distinguish between these two processes using an S1-myosin heptapeptide inhibitor of the actomyosin interaction is problematic (Suzuki *et al.*, 1987; Eto *et al.*, 1991). Our BDM data argue against a role for myosin II in generating force for retraction of the cell margin during rounding of cells at mitosis. Morphologically, mitotic rounding cells are similar to cells undergoing apoptosis or oncogenic transformation; therefore, it is possible that myosin II similarly does not play a role in force generation during rounding of these cell types.

In lamellipodia, actin-based rearward flow of receptors, surface-attached beads, and cytoplasmic structures have been studied for decades. No one mechanism for rearward flow in lamellipodia can account for all of the experimental data. There is strong evidence, mostly from *Aplysia* growth cones, that this flow is driven by moving actin filaments (Forscher and Smith, 1990; Lin and Forscher, 1995) and, from BDM inhibition, that the actin filament movement depends on myosin activity (Lin *et al.*, 1996). However, rearward flow of cell structures in keratocyte and tissue culture fibroblast lamellipodia appears to be more complex (from data presented in Kucik *et al.*, 1990, 1991; Theriot and Mitchison, 1991, 1992b). This suggests the existence of a second force for rearward flow in lamellipodia outside the *Aplysia* system. Some flowing structures resemble nodules. We propose that a second force for rearward flow in lamellipodia is generated by a pointed end-directed actin-based motor. Clearly, any acceptance of this idea will hinge on identifying such a motor, an experimental direction we plan to take in the future.

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REFERENCES

Chen, W.-T. (1981). Mechanism of retraction of the trailing edge during fibroblast movement. J. Cell Biol. *90*, 187–200.

Cramer, L.P., and Mitchison, T.J. (1993). Moving and stationary actin filaments are involved in spreading of postmitotic PtK2 cells. J. Cell Biol. *122*, 833–843.

Cramer, L.P., and Mitchison, T.J. (1995). Myosin is involved in postmitotic cell spreading. J. Cell Biol. *131*, 1–11.

Cramer, L.P., Mitchison, T.J., and Theriot, J.A. (1994). Actin-dependent motile forces and cell motility. Curr. Opin. Cell Biol. *6*, 82–86.

Croop, J., and Holtzer, H. (1975). Response of myogenic and fibrogenic cells to cytochalasin B and to Colcemid. J. Cell Biol. *65*, 271–285.

Crowley, E., and Horwitz, A., F. (1995). Tyrosine phosphorylation and cytoskeletal tension regulate the release of fibroblast adhesions. J. Cell Biol. *131*, 525–537.

Eto, M., Morita, F., Nishi, N., Tokura, S., Ito, T., and Takahashi, K. (1991). Actin polymerization promoted by a heptapeptide, an analog of the actin-binding S site on myosin head. J. Biol. Chem. *266*, 18233–18236.

Forscher, P., and Smith, S.J. (1990). Cytoplasmic actin filaments move particles on the surface of a neuronal growth cone. In: Optical Microscopy for Biology, New York: Wiley-Liss, Inc.

Fukui, Y., De Lozanne, A., and Spudich, J.A. (1990). Structure and function of the cytoskeleton of a Dictyostelium myosin-defective mutant. J. Cell Biol. *110*, 367–378.

Grebecki, A. (1994). Membrane and cytoskeletal flow in motile cells with emphasis on the contribution of free-living amoeba. Int. Rev. Cytol. *148*, 37–80.

Harris, A.K. (1973). Location of cellular adhesions to solid substrata. Dev. Biol. *35*, 97–114.

Higuchi, H., and Takemori, S. (1989). Butanedione monoxime suppresses contraction and ATPase activity of rabbit skeletal muscle. J. Biochem. *105*, 638–643.

Kucik, D.F., Elson, E.L., and Sheetz, M.P. (1990). Cell migration does not produce membrane flow. J. Cell Biol. *111*, 1617–1622.

Kucik, D.F., Kuo, S.C., Elson, E.L., and Sheetz, M.P. (1991). Preferential attachment of membrane glycoproteins to the cytoskeleton at the leading edge of lamella. J. Cell Biol. *114*, 1029–1036.

Lin, C.-H., and Forscher, P. (1995). Growth cone advance is inversely proportional to retrograde F-actin flow. Neuron *14*, 763–771.

Lin, C.-H., Espreafico, E.M., Mooseker, M.S., and Forscher, P. (1996). Myosin drives retrograde F-actin flow in neuronal growth cones. Neuron *16*, 769–782.

Mitchison, T.J. (1992). Actin based motility on retraction fibers in mitotic PtK2 cells. Cell Motil. Cytoskeleton *22*, 135–151.

Pasternak, C., Spudich, J.A., and Elson, E.L. (1989). Capping of surface receptors and concomitant cortical tension are generated by conventional myosin. Nature *341*, 549–551.

Ramsey, W.S. (1972). Locomotion of human polymorphonuclear leucocytes. Exp. Cell Res. *72*, 489–501.

Sanger, J.M., Reingold, A.M., and Sanger, J.W. (1984). Cell surface changes during mitosis and cytokinesis of epithelial cells. Cell Tissue Res. *237*, 409–417.

Sanger, J.W., and Sanger, J.M. (1992). Beads, bacteria and actin. Nature *357*, 442.

Satterwhite, L.L., Lohka, M.J., Wilson, K.L., Scherson, T.Y., Cisek, L.J., Corden, J.L., and Pollard, T.D. (1992). Phosphorylation of myosin-II regulatory light chain by cyclin-$p34^{cdc2}$: a mechanism for the timing of cytokinesis. J. Cell Biol *118*, 595–605.

Sheetz, M.P. (1993). Glycoprotein motility and dynamic domains in fluid plasma membranes. Annu. Rev. Biophys. Biomol. Struct. *22*, 417–431.

Sheetz, M.P., Turney, S., Qian, H., and Elson, E.L. (1989). Nanometre-level analysis demonstrates that lipid-flow does not drive membrane glycoprotein movements. Nature *340*, 284–288.

Sims, J.R., Karp, S., and Ingber, D., E. (1992). Altering the cellular mechanical force balance results in integrated changes in cell, cytoskeletal and nuclear shape. J. Cell Sci. *103*, 1215–1222.

Spooner, B.S., Yamada, K.M., and Wessells, N.K. (1971). Microfilaments and cell locomotion. J. Cell Biol. *49*, 595–613.

Suzuki, R., Nishi, N., Tokura, S., and Morita, F. (1987). F-actin-binding synthetic heptapeptide having the amino acid sequence around the SH1 cysteinyl residue of myosin. J. Biol. Chem. *262*, 11410–11412.

Theriot, J.A., and Mitchison, T.J. (1991). Actin microfilament dynamics in locomoting cells. Nature *352*, 126–131.

Theriot, J.A., and Mitchison, T.J. (1992a). The nucleation-release model of actin filament dynamics in cell motility. Trends Cell Biol. *2*, 219–222.

Theriot, J.A., and Mitchison, T.J. (1992b). Comparison of actin and cell surface dynamics in motile fibroblasts. J. Cell Biol. *118*, 367–377.

Wetzel, B., Kendig, E.M., Jones, G.M., and Sanford, K.K. (1978). A systematic scanning electron microscope (SEM) analysis of mitotic cell populations in monolayer culture. Scanning Electron Microsc. *2*, 1–10.

Yamada, K.M., and Miyamoto, S. (1995). Integrin transmembrane signaling and cytoskeletal control. Curr. Opin. Cell Biol. *7*, 681–689.

Yamakita, Y., Yamashiro, S., and Matsumura, F. (1994). In vivo phosphorylation of regulatory light chain of myosin II during mitosis of cultured cells. J. Cell Biol. *124*, 129–137.