Coordination of Protrusion and Translocation of the Keratocyte Involves Rolling of the Cell Body

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Abstract. We have investigated the relationship between lamellipodium protrusion and forward translocation of the cell body in the rapidly moving keratocyte. It is first shown that the trailing, ellipsoidal cell body rotates during translocation. This was indicated by the rotation of the nucleus and the movement of cytoplasmic organelles, as well as of exogenously added beads used as markers. Activated or Con A–coated fluorescent beads that were overrun by cells were commonly endocytosed and rotated with the internal organelles. Alternatively, beads applied to the rear of the cell body via a micropipette adhered to the dorsal cell surface and also moved forward, indicating that both exterior and underlying cortical elements participated in rotation. Manipulation of keratocytes with microneedles demonstrated that pushing or restraining the cell body in the direction of locomotion, and squeezing it against the substrate, which temporarily increased the intracellular pressure, did not effect the rate of lamellipodium protrusion. Rotation and translocation of the cell body continued momentarily after arrest of lamellipodium protrusion by cytochalasin B, indicating that these processes were not directly dependent on actin polymerization.

The cell body was commonly flanked by phase-dense "axles," extending from the cell body into the lamellipodium. Phalloidin staining showed these to be comprised of actin bundles that splayed forward into the flanks of the lamellipodium. Disruption of the bundles on one side of the nucleus by traumatic microinjection resulted in rapid retraction of the cell body in the opposite direction, indicating that the cell body was under lateral contractile stress. Myosin II, which colocalizes with the actin bundles, presumably provides the basis of tension generation across and traction of the cell body.

We propose that the basis of coupling between lamellipodium protrusion and translocation of the cell body is a flow of actin filaments from the front, where they are nucleated and engage in protrusion, to the rear, where they collaborate with myosin in contraction. Myosin-dependent force is presumably transmitted from the ends of the cell body into the flanks of the lamellipodium via the actin bundles. This force induces the spindle-shaped cell body to roll between the axles that are created continuously from filaments supplied by the advancing lamellipodium.

T HE crawling of cells over substrata generally involves protrusion of the front followed by retraction of the rear. In fibroblasts, this is a particularly erratic, apparently uncoordinated process (Dunn, 1980; Chen, 1981; Trinkaus, 1984), whereas in leukocytes and some amoebae, the cell rear makes a more concerted effort to keep up with the front (Lackie, 1986; Fukui, 1993). These differences can be correlated with variations in the contact-making machinery (Abercombie and Dunn, 1975; Izzard and Lochner, 1976; King et al., 1980; Gingell and Vince, 1982) with the slow-moving fibroblasts exhibiting the strongest contacts, which less willingly yield to the

cells' protrusive intentions. Both protrusion and retraction use actin filaments as their structural elements, but protrusion involves a dynamic polymerization process (Smith, 1988; Mitchison and Kirschner, 1988; Zigmond, 1993; Small, 1989, 1995); on the other hand, retraction is apparently based on contractile activity (Abercrombie et al., 1970), dependent on conventional, filament-forming myosin II (Chen, 1981; Fukui et al., 1989; Small, 1989; Jay et al., 1995). However, the molecular details of the two processes, and how and to what extent they are coordinated, are still fragmentary.

The epidermal keratocyte, derived from fish or amphibia (Goodrich, 1924; Bereiter-Hahn et al., 1981), is marked for its rapid locomotion among those cells that use actin to crawl. Unlike other cells, in which protrusion of the lamellipodium is interspersed with phases of arrest,

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withdrawal, and ruffling (Abercombie et al., 1970; Trinkaus, 1984), the keratocyte is able to protrude its lamellipodium continuously. Since the rate of lamellipodium protrusion corresponds to the speed of locomotion of the whole cell, the large trailing cell body must keep pace with the lamellipodium, indicating a tightly regulated link between protrusion and retraction. The question arises as to how the cell body, which makes up 95% of the cell volume, is drawn forward with the lamellipodium. Is it tethered to the rear of the lamellipodium and transported by endoplasmic contraction (Bereiter-Hahn et al., 1981; Sheetz, 1994) or are forces exerted, for example, to the sides or to the rear of the cell body (Lee et al., 1994) responsible for its forward translocation? And how does the cell body move over the substrate so as to provide the least resistance to the protrusion of the cell front?

It was at first somewhat of a surprise to find, as we demonstrate in the present study, that the cell body of the rapidly moving keratocyte, in fact, is able to roll along behind the lamellipodium. By adopting this method, the cell has avoided the problem of having to overcome the much higher frictional forces involved in sliding its rear end. In addition to documenting this behavior, we show, using micromanipulation, that neither increased cytoplasmic pressure nor positive or negative displacement of the cell body in the direction of motion influences the rate of lamellipodium protrusion. The data are discussed in terms of a model of keratocyte locomotion in which the forward translocation of the cell body is coordinated with protrusion in the lamellipodium via a flow of actin filaments.

Materials and Methods

Culture of Keratocytes

Fish epidermal keratocytes were cultured according to a procedure modified from Small et al. (1995). Briefly, scales were harvested from the fish into DMEM, rinsed twice, transferred to "start medium" (see Small et al., 1995), and pressed between two coverslips in a drop of start medium. Cells in this condition attached to the coverslip and formed monolayer halos around the scale, and could be kept in humidity chambers at 4°C for up to 4 d. On the day of use, coverslips were affixed to the bottom of observation chambers (a 50-mm petri dish with an 8-mm hole in the bottom) with silicon vacuum grease, and the chamber was flooded with start medium containing 0.25 mg/ml collagenase for 15–30 min to dissociate the cells. Running buffer (see Small et al., 1995) was then added to the mixture in the chamber, and the cells were returned to the refrigerator for an additional 2 h or more until use.

Microscopy

Keratocytes were observed at room temperature on an inverted microscope (Axiovert 135TV; Carl Zeiss, Vienna, Austria) equipped for epifluorescence, phase-contrast, and differential-interference contrast microscopy, using ×40/NA 0.66 Achroplan LD or ×100/NA 1.3 Plan-Neofluar objectives and up to ×2.5 optovar intermediate magnification. Images were acquired using one of two systems: phase-contrast and differential-interference contrast images were collected via a camera (240077 CCD; Hamamatsu GmbH, Herrsching, Germany) coupled to an Argus 10 image processor, and recorded in real time using a video recorder (Umatic; Sony, Vienna, Austria). Video frames were later digitized using a frame grabbing card (LG-3; Scion Corp., Frederick, MD), and analyzed on a Macintosh Power PC7100/80 using NIH Image (Apple Computer Co., Cupertino, CA). Low light fluorescence, as well as simultaneous phase and fluorescent images, were acquired and stored as 16-bit digital sequences using a back-illuminated cooled CCD camera from Princeton Research Instruments, Inc. (Princeton, NJ) driven by IPLabs software (both from Visitron Systems, Eichenau, Germany).

Micromanipulation

For manipulations of the cell body, thin microneedles were pulled using a needle puller (Narishiga, Tokyo, Japan) and briefly passed near a flame to blunt them. For the application of latex beads to individual keratocytes, microneedles were pulled, the tip was broken, and the jagged edge was rounded by passing near a flame. This resulted in a large round hole, through which a solution of latex beads flowed smoothly. Traumatic microinjections of keratocytes were performed with conventional needles by injecting BSA (Sigma, Vienna, Austria) at higher than normal needle pressure (50 hPa) using a pressure regulator (Eppendorf, Hamburg, Germany). In all cases, a Leitz Micromanipulator M (Leica, Vienna, Austria) was used to control the microneedles.

Vital Staining and Other Procedures

A stock of rhodamine 123 (Sigma) was prepared in 50% ethanol in PBS. For staining, cells were incubated with the dye at a concentration of 10 μg/ml in Ca and Mg-free running buffer for 10 min at room temperature, and then rinsed in running buffer and kept at 4°C until viewing on the microscope.

Con A coating of 0.5-μm-diam rhodamine-labeled Covaspheres (Duke Scientific Corp., Palo Alto, CA) was performed according to Theriot and Mitchison (1992), including glutaraldehyde activation. Covaspheres have activated surface groups that react automatically with proteins; when diluted straight from the manufacturer's stock, they are referred to in the text as activated beads. Covaspheres were generally applied to the cells at a 1:10,000 dilution in running buffer and incubated for 15 min. The beads were rinsed out, and the cells returned to 4°C until viewing. For the direct application of beads to the rear of a cell, they were diluted 1:100 in running buffer and loaded into an application needle. The pressure regulator was set for 1 hPa, and the needle was brought to a position near the substrate behind the cell, so that beads flowing out of the needle struck the cell's rear and adhered to it.

A stock 1 mg/ml solution of cytochalasin B (Sigma) was prepared in DMSO and used at a final concentration of 400 ng/ml in running buffer.

Immunofluorescence and EM

Keratocytes were fixed for immunofluorescence microscopy in 3% paraformaldehyde in PBS for 30 min followed by extraction in 0.5% Triton X-100 in PBS for 30 s. The antibody against myosin subfragment-1 (S-1) was raised in rabbits against chicken gizzard myosin S-1, and affinity purified on a column carrying the antigen. Phalloidin staining was accomplished simultaneously with antibody incubation, not overnight, as described previously (Small et al., 1995).

For EM, cells on glass coverslips were fixed in a mixture of 2.5% glutaraldehyde, 2% paraformaldehyde, and 0.2% tannic acid in PBS for 1 h at room temperature. They were rinsed in the same buffer overnight, postfixed in 1% OsO₄ in PBS for 1 h at room temperature, blockstained in aqueous uranyl acetate, dehydrated in alcohol, and embedded in Araldite. After polymerization, the coverslips were removed using fluoric acid, and a thin Araldite layer was cast over the exposed cell layers to offer protection during trimming and thin sectioning. Thin, silver-gold sections were contrasted with aqueous uranyl acetate and lead citrate and viewed in an electron microscope (EM/10A; Carl Zeiss, Inc.).

Results

The Keratocyte Cell Body Rolls

During the observation of time-lapse video sequences of locomoting keratocytes in phase contrast (×40 objective), light and dark particles that moved in opposite directions relative to each other as the cell moved were seen in the cell body (Fig. 1 a). Focusing up and down in the cell established that the dark particles were ventral and the light particles were dorsal. Since the same group of dark particles on the ventral surface reappeared as light particles on the dorsal surface, it was clear that the cell body was rotating during cell locomotion. To establish to what extent this rotation involved other organelles, we vitally labeled mito-
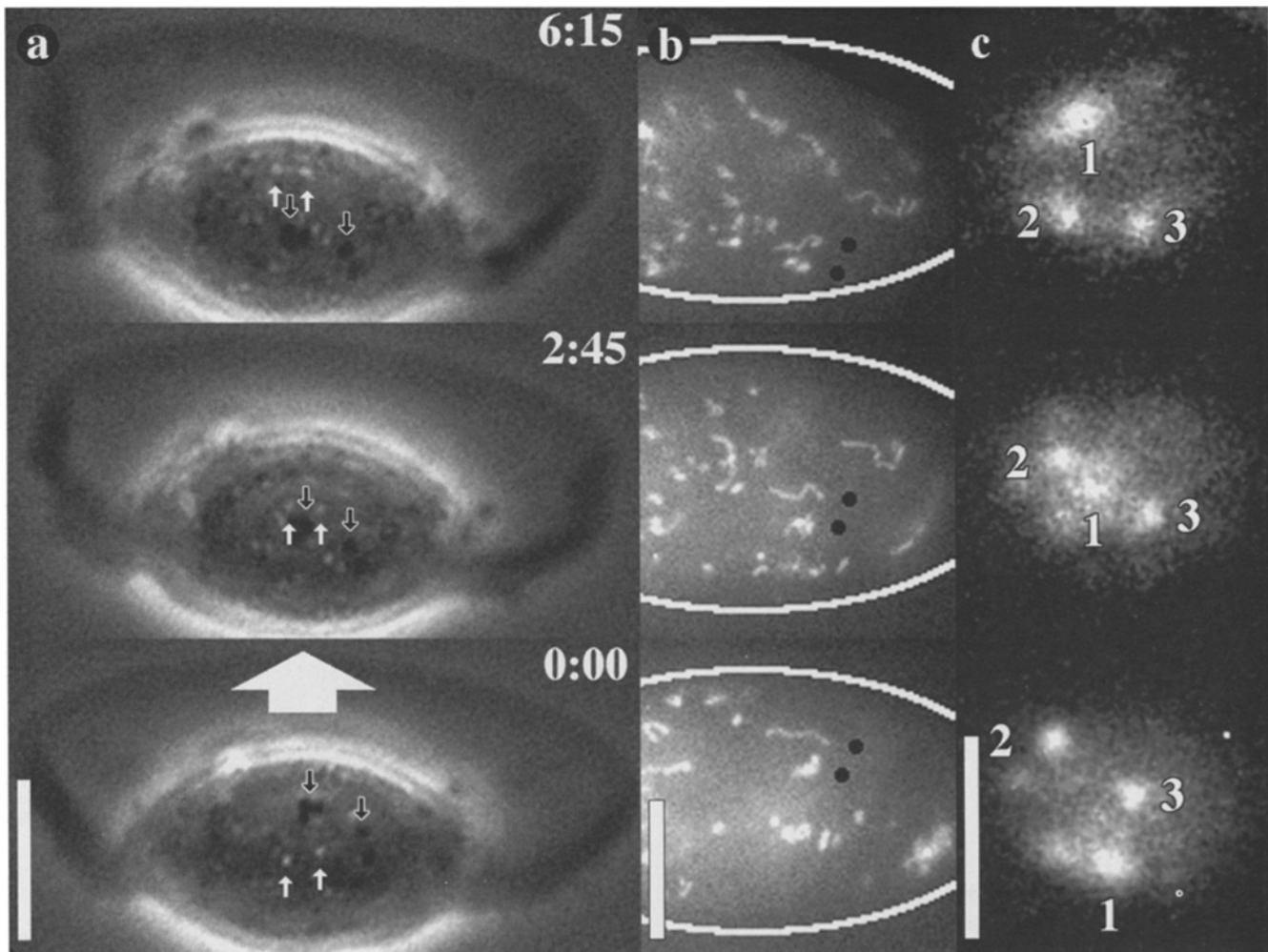
Figure 1. (*a*) Three video frames of a moving keratocyte taken using phase-contrast optics (×40 objective) at the times given, in min and s. Particles in the cell body appear dark when close to the substrate, and light when close to top of the cell body. Two particles of each type are highlighted by the placement of black or white arrows, respectively. Note that the two sets of particles move relative to each other, consistent with a rolling motion. (*Large arrow*) Direction of locomotion in *a*, *b*, and *c*. (*b*) Fluorescent images of a cell incubated with rhodamine 123 to mark mitochondria and recorded using a ×100 objective for which only the ventral cell surface was in focus. Black spots mark two mitochondria that move backwards relative to the cell body. (*c*) Autofluorescence of nuclear structures in a moving keratocyte. The same three structures are numbered in each subsequent video image. Structures 2 and 3 move in the subsequent frames from the front of the cell body to the back; structure 1 moves from back to front, consistent with rotation of the cell body. The full video sequences document the rotation in each case more dramatically. Bars, 10 μm.

chondria with rhodamine 123. Owing to their small size, the mitochondria were best visualized at higher magnification (×100 lens), at which the depth of focus was limited to either the dorsal or ventral part of the cell body. In this case, movement of mitochondria consistent with their translocation around the cell body was also recorded (Fig. 1 *b*). At very low illumination intensities, unlabeled cells exhibited autofluorescence of particulate bodies in the nucleus that became bleached at high intensities. These gave us the opportunity to follow changes in nuclear orientation, which again complied with a rotational movement accompanying locomotion (Fig. 1 *c*).

In cross-sections of embedded cells, the profile of the fish keratocytes was compared with that previously shown for keratocytes from *Xenopus* (Bereiter-Hahn et al., 1981): the cell body was approximately circular in form and was connected at its base to the thin lamellipodium

sheet of 0.2–0.3 μm in thickness (Fig. 2 *a*). Lipid droplets were commonly observed in the perinuclear region and presumably corresponded to the particles seen by phase-contrast microscopy. Sectioning of cells treated with latex beads revealed that many were endocytosed by the cell and became situated in the perinuclear cytoplasm (Fig. 2 *b*).

Exogenously Applied Beads Rotate with the Cell Body

When activated or Con A–coated beads were added in bulk to the medium of a cell culture, many adhered to the substrate and were subsequently picked up by the cells that moved over them. For these beads, two characteristic types of behavior were observed. In one case, beads were overrun by the cells, and then were lifted off the substrate when they reached the rear of the cell body. Such beads were presumably endocytosed (Fig. 2 *b*), appeared to be
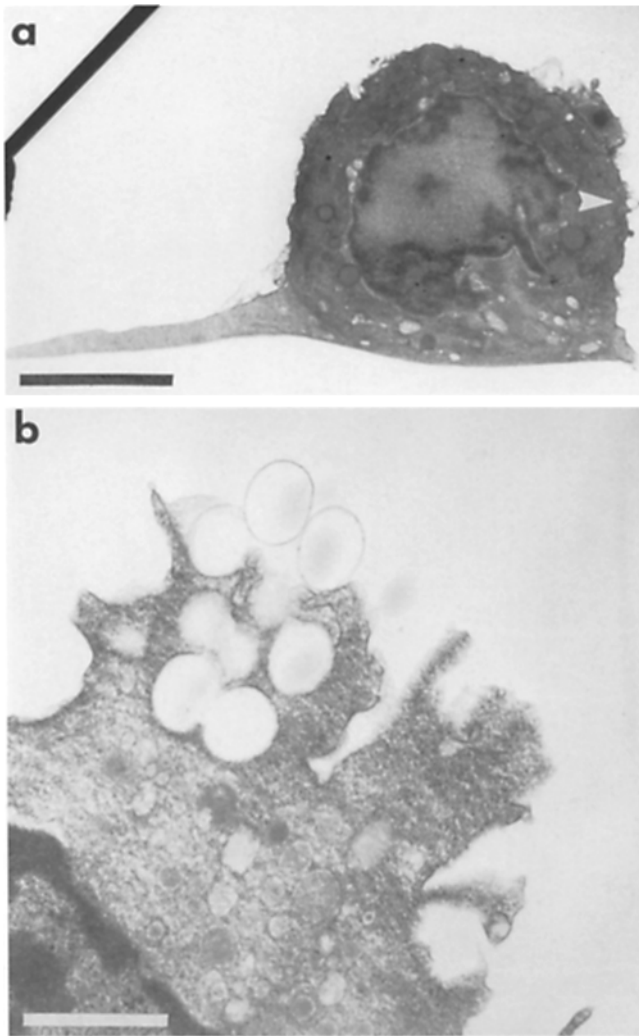
Figure 2. (*a*) Electron micrograph of a cross-section through a keratocyte showing approximately circular cell body profile and the projecting lamellipodium. (*Arrowhead*) Bead that has attached to the cell body surface. (*b*) Higher magnification view of a cell that had been incubated with 0.5-μm Covaspheres for several hours. A large group of beads is attached to the cell body surface, and several beads have already been engulfed by the cell. Bars: (*a*) 5 μm; (*b*) 1 μm.

on the inner face of the cell membrane, and were observed to make complete rotations in the cell body (Fig. 3).

In the second case, beads were dislodged from the substrate by the lamellipodium and moved onto its dorsal surface. These beads either moved randomly or were rapidly transported to the rear of the lamellipodium, in agreement with the findings of Kucik et al. (1990). Those that moved to the back of the lamellipodium were seen to track along the border with the cell body (Fig. 4 *a*). These beads eventually fell off at the far ends of the cell body or made cyclic loops around its tapered end, moving to the rear and then over the cell body again.

To follow surface movements on the cell body, we applied beads to the rear of keratocytes via a micropipette. Activated beads adhered readily to the rear of the cell, whereas Con A–coated beads bound less frequently. Once
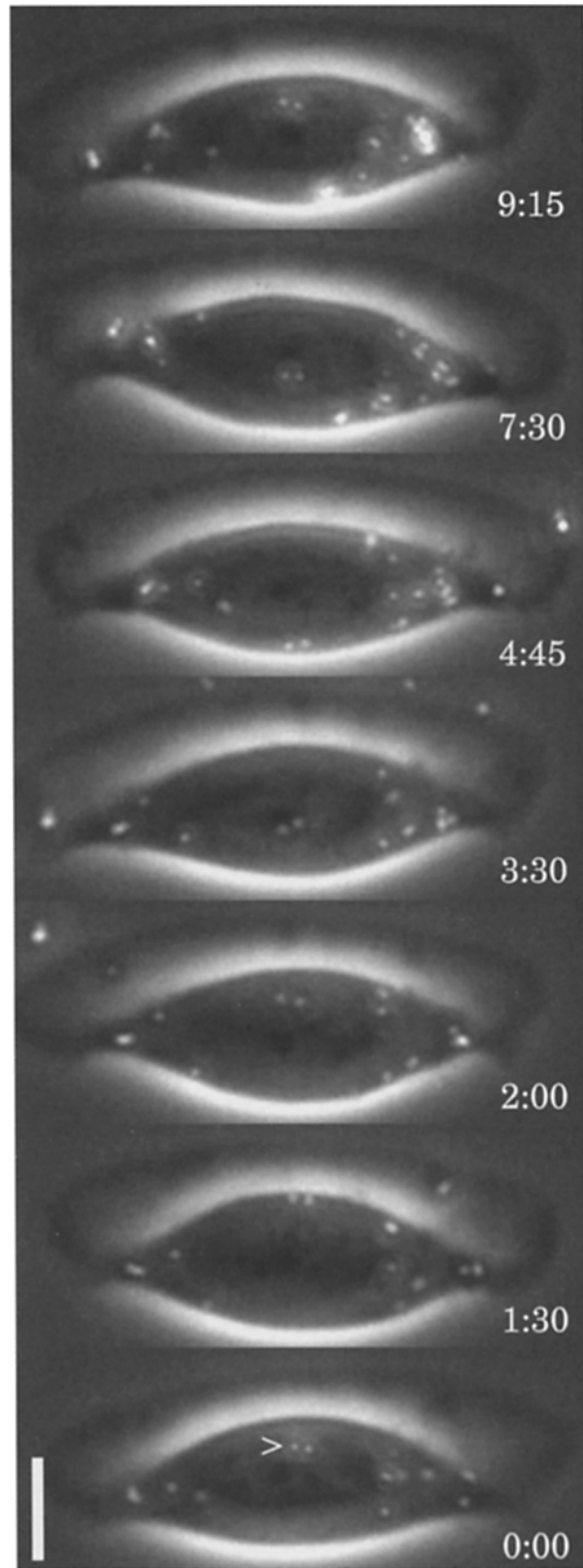
Figure 3. Movement of fluorescent beads that were incorporated into the cell body. Series shows two beads (*arrow at 0:00*) that were overrun by the cell and engulfed before the shown sequence. Note that the beads make a complete rotation around the cell body and remain within its boundaries at all times. Time at bottom right is in min and s, indicating also distance moved at 10 μm/min; total distance moved in the sequence was 95 μm. Bar, 10 μm.

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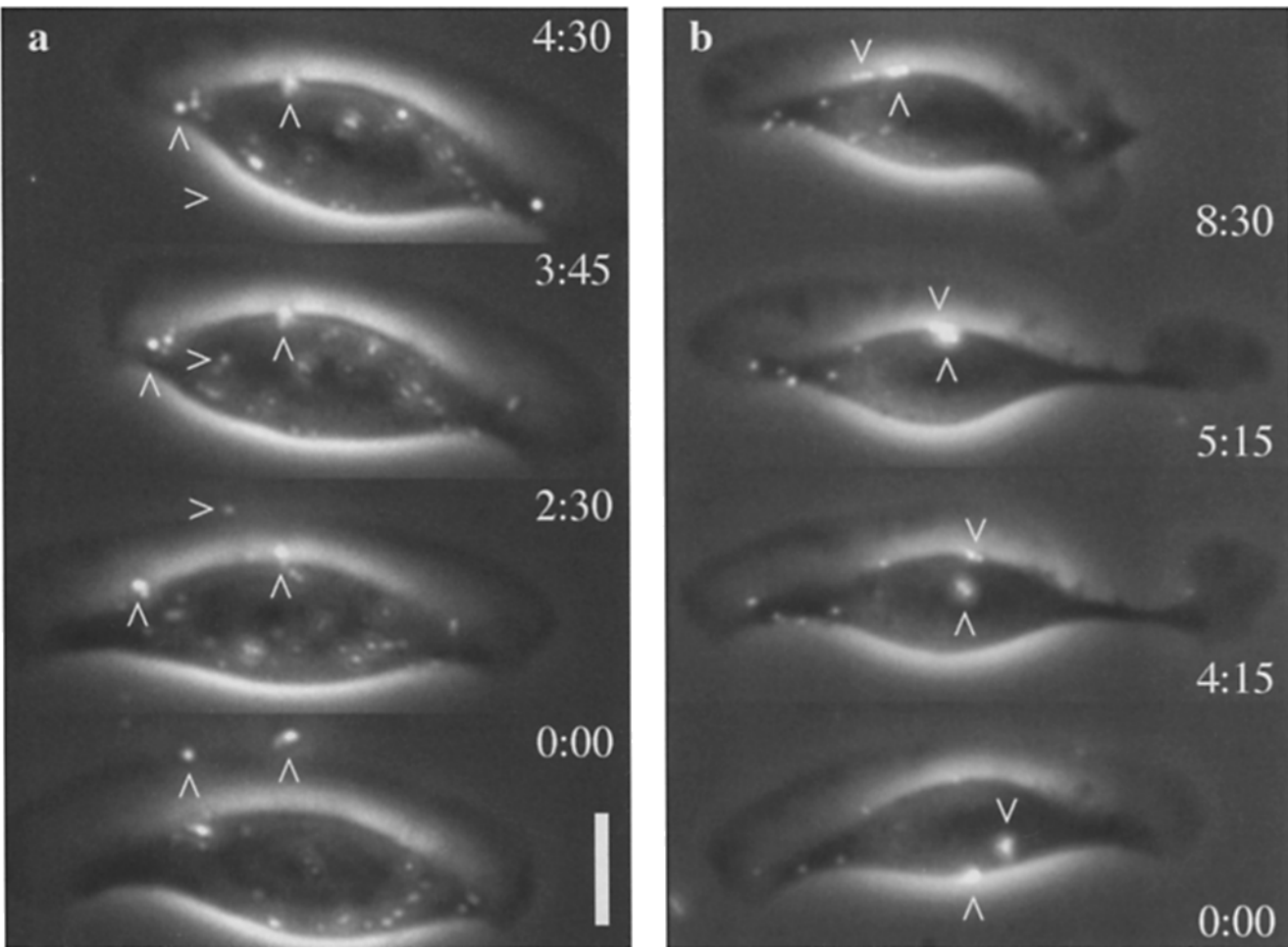
Figure 4. Movement of fluorescent beads attached to the outer cell surface. (*a*) Two beads on the substrate (*arrowheads at 0:00*) were picked up at the front of the lamellipodium. They both diffused rearward to the border of the cell body, where they then moved laterally to the far edge. (*Horizontal arrowheads*) Position of a bead that was overlapped by the lamellipodium and picked up under the cell body (*3:45*), as shown by its subsequent absence from the substrate (*4:30*). (*b*) Beads (*arrowheads*) were applied with a micropipette directly to the rear surface of the cell; the rearmost bead is seen to be outside the cell body. Both remained attached until they reached the front of the cell body, where they migrated laterally along the boundary with the lamellipodium. Bar, 10 μm.

attached, both types of bead rotated up and over the cell body (Fig. 4 *b*), at the same rate as endocytosed beads already within the cell. Significantly, externally applied beads that followed this route moved sideways when they reached the forward boundary of the cell body with the lamellipodium (Fig. 4 *b*), in the same way as beads that approached this boundary from the front (see above and Fig. 4 *a*), but in contrast to the endocytosed beads (Fig. 3).

Using the surface-bound and endocytosed beads as markers, we compared the rate of rotation of the cell body with the distance traveled. For the typical example shown in Fig. 3, the distance covered by the cell to produce one rotation of the cell body was 95 μm (cell velocity was 10 μm/min). Given a cell body diameter of ~10 μm, three rotations would have been possible, indicating that there was slippage between the cell body and the substrate. If there were no slippage, we would expect beads on the upper cell body surface to move twice as fast as the cell. Measurements of the displacement of 13 beads that moved over the central region of 10 different cells gave an average velocity of 1.48 (± 0.13) times the locomotion velocity. Corre-

spondingly, beads on the ventral surface slipped forward over the substrate at about half the speed of cell movement.

Protrusion and Translocation Are Indirectly Coupled

To assess the degree of interdependence of lamellipodium protrusion and cell body rolling, microneedles were used to exert force on the cell body, while simultaneously monitoring the rate of advance of the front of the lamellipodium (Fig. 5). Fig. 5, *a* and *b*, show situations in which the body of a keratocyte was either pushed in the rear by a microneedle (Fig. 5 *a*) or temporarily restrained from advancing with the cell front (Fig. 5 *b*). In the frames that are equally spaced in time, white lines mark the positions of the front edge of the lamellipodium, with the last frames showing all three positions. As can be seen, gross distortions in cell body position had no effect on the rate of protrusion of the cell front. A third manipulation involved the application of continuous pressure to the cell body (for up to 30 s), by pressing a blunted needle down onto it (Fig. 5
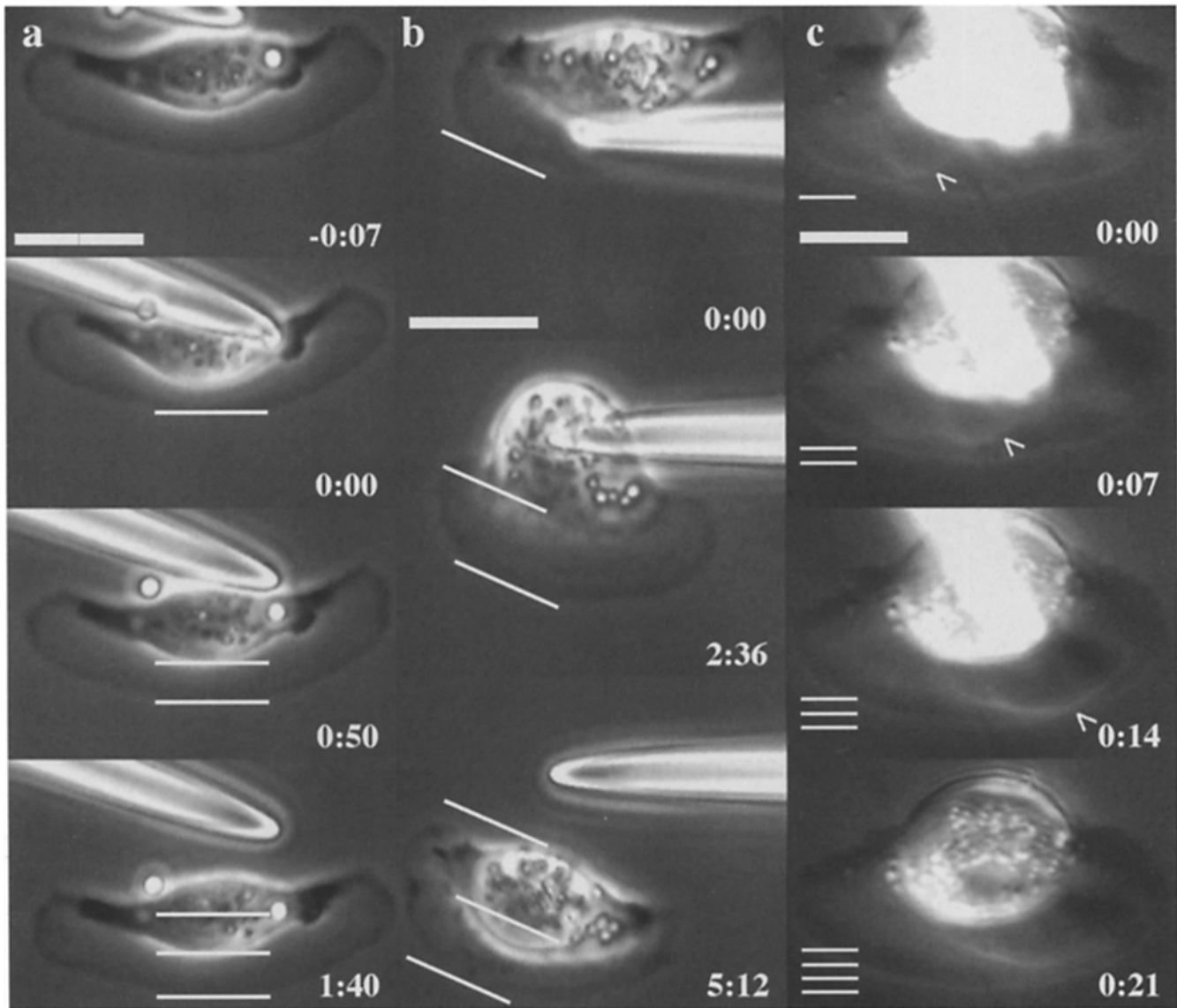
Figure 5. Mechanical manipulation of the cell body. (*a*) Video sequence showing result of pushing cell body from the rear. (*White lines*) Position of front of lamellipodium in subsequent frames. At −0:07, the needle is positioned behind the cell; at 0:00, it has been thrust 5 μm forward. The time difference between each of the last three frames is the same, as is also the difference in displacement of the lamellipodium. (*b*) Restraint of cell body by glass needle. Otherwise as for *a*. Protrusion rate of lamellipodium is unaffected, as shown by equidistant lines at 5:12. (*c*) Result of squeezing cell body from above with glass needle. (*Arrow*) Bulge, which spreads across the lamellipodium from left to right in the first three frames, although the rate of advance of the front edge, marked to the left by cumulative white lines from frame to frame, is unaffected. (*a* and *b*) Phase contrast optics; (*c*) Normarski optics. Bars, 10 μm.

c). By this means, increases in cytoplasmic pressure were induced, as evidenced from the appearance of blisters in the lamellipodium (Fig. 5 *c, arrow*). Despite this rather drastic treatment, which, however, could be repeated on the same cell, the rate of advance of the cell edge was unaffected.

We then treated cells with limiting concentrations of cytochalasin B that caused a rapid cessation of protrusion of the cell front, without withdrawal in the short term. As shown in Fig. 6, arrest of lamellipodium protrusion did not cause a simultaneous arrest of rolling, which continued until the front edge of the cell body had advanced halfway across the lamellipodium. This showed that cell body translocation was not immediately dependent on a process based on actin polymerization.

The lack of coupling between central regions of the cell body and the lamellipodium was further supported by transient morphologies of keratocytes, such as shown in Fig. 7. During the course of normal movement, cells that resorbed the central region of their lamellipodium and moved temporarily using only lamellipodium flanks could be found. In preparations incubated with fluorescent beads, we could show that cell body rotation continued in this condition (Fig. 7).

The Spindle Shape of the Cell Body Is Maintained by Lateral Tension

Double labeling of keratocytes with an antibody against

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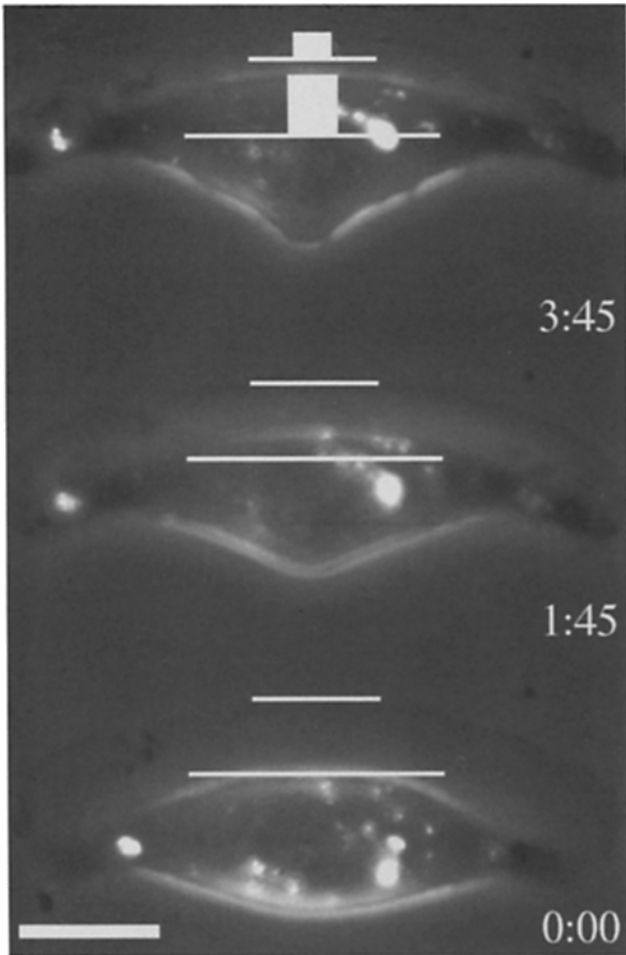
Figure 6. Cytochalasin treatment. At 400 ng/ml cytochalasin, the lamellipodium was rapidly arrested (*3:45*), but the cell body continued to move forward. The two white lines mark the initial positions of the cell front and the front boundary of cell body. (*Bars at 3:45*) Total distances moved by each in the sequence. Bar, 10 μm.

myosin II and rhodamine-phalloidin revealed that the motor molecule was concentrated around the cell body and colocalized with bundles of laterally oriented actin filaments, which were commonly found to link the ends of the cell body with the flanks of the lamellipodium (Fig. 8). The average angle of bundle orientation for 17 cells was 7.8° forward of the lateral axis. Optical sectioning indicated that the actin bundles were not restricted to a plane near the substrate, but they also extended up around the cell body (data not shown). Myosin II was excluded from the anterior regions of the lamellipodium. The concentration of myosin II around the cell body suggested that it could serve to provide the lateral tension necessary for maintaining its spindle-like shape. Evidence for this was obtained by traumatic microinjection experiments. Keratocytes were microinjected with BSA at higher than normal needle pressures, which resulted in the local disruption of the plasma membrane and cytoskeleton at one end of the cell body, corresponding in position to the lateral actin bundles. This manipulation typically resulted in a rapid retraction of the cell body toward the opposite end (Fig. 9), sug-
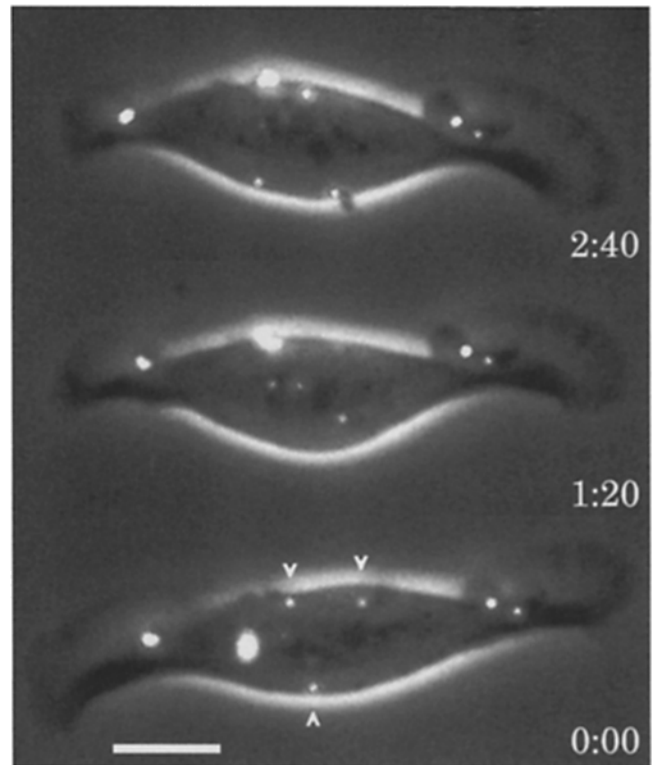

Figure 7. Cell in a phase of locomotion involving only lateral lamellae. Beads move in the direction indicated by arrows in 0:00. Note the pattern inversion between 0:00 and 2:40, indicating that the cell body also rotates in this morphology. Bar, 10 μm.

gesting the release of lateral tension. Frequently, this treatment was nonlethal; cells simply resorbed their trailing remnants and veered off in a slightly different direction.

Discussion

Our observations of phase-dense bodies, nuclear markers, and vitally labeled mitochondria show that the cytoplasm within the keratocyte cell body rotates during locomotion. Furthermore, Con A–coated beads applied to the cell exterior moved forward in a directed manner over the cell body at the same rate as endocytosed beads, indicating the linkage of the former to internal, cytoskeletal elements (Kucik et al., 1989, 1990). Since the cell body of the keratocyte rotates continuously as it moves, there cannot be more than a transient connection of structural elements between its front border and the lamellipodium. Forward translocation of the cell body is therefore unlikely to occur via a frontal connection into the lamellipodium through a cortical or endoplasmic network of contractile filaments (Sheetz, 1994; Bray and White, 1988). As we show, the primary structural connection between the lamellipodium and the cell body is at the flanks of the lamellipodium, where actin filament bundles accumulate. The major contractile stress does not exist in the direction of locomotion, but is instead directed laterally across the cell body. This is shown from the present microdissection experiments and from the work of Lee et al. (1994), who monitored the forces developed by keratocytes moving on flexible substrata. It has formerly been speculated that contractile
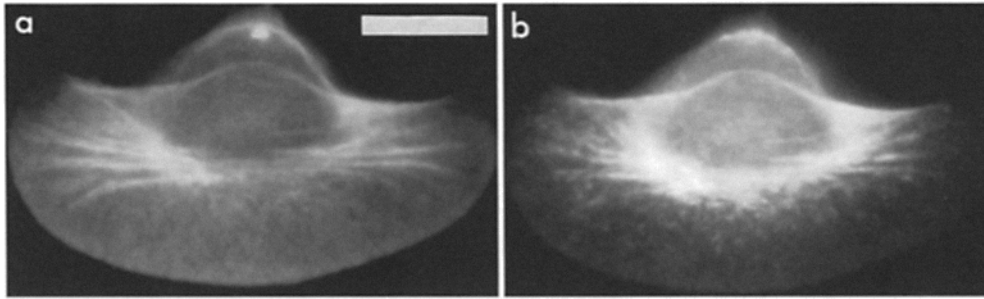
Figure 8. Immunofluorescence images of a cell double labeled with phalloidin (*a*) and antibodies to myosin (*b*). Bar, 10 μm.

stress around the keratocyte cell body and at the rear of the lamellipodium may produce the driving force behind lamellipodium protrusion, via increased hydrostatic pressure (Bereiter-Hahn et al., 1981). We have excluded this possibility by showing that squeezing of the cell body has no effect on the rate of lamellipodium advance. This finding underlines the conclusion that contractile activity is not required for protrusion, as has been demonstrated
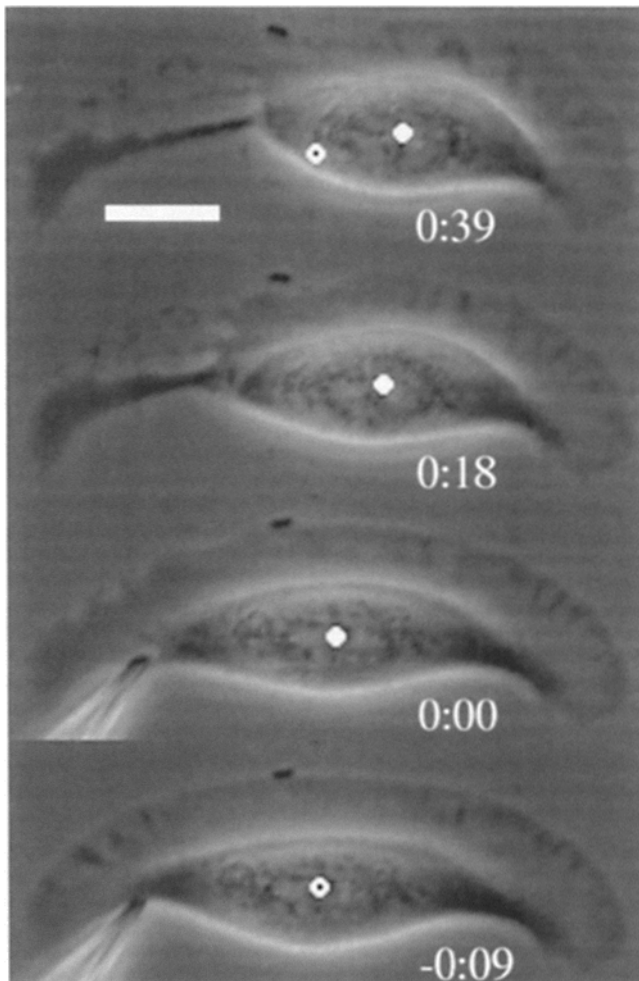
Figure 9. Traumatic microinjection at the end of the cell body (*0:00*) causes local disruption of the cytoskeleton and retraction of the cell body toward the opposite side. (*White spots*) Center of the cell body; (*white spot with black point*) position before injection, at time −0:09. Bar, 10 μm.

most convincingly in *Dictyostelium* myosin II null mutants that are still competent to protrude lamellipodia (Fukui et al., 1990).

Euteneuer and Schliwa (1984) were the first to observe that fragments of lamellipodia derived from keratocytes can migrate as fast as the parent cell. We demonstrate here that forward thrusting of the cell body, or its restraint by a microneedle, does not affect the rate of lamellipodium protrusion. Thus, in terms of motility, the cell body appears to be excess baggage, not contributing to forward protrusion in any way (Heath and Holifield, 1991). But to produce as little hindrance to locomotion as possible, the cell body has been coupled with its "front-wheel-drive" lamellipodium, in a manner that minimizes the energy of its translocation and entails a tight coordination of events between the front and the rear of the cell. We propose that the dynamics of actin filament turnover forms the basis of this coordination. Our model, summarized in Fig. 10, is an extension of earlier ideas about the lateral flow of actin filaments in the lamellipodium (Small et al., 1993; Small, 1994, 1995). It further incorporates the existence of a cortical cage of actin filaments around the cell body whose presence is supported by a delimitation of the cell body by a surface layer of phalloidin-positive material (Fig. 8; Small et al., 1995).

The first assumption we make is that the nucleation of actin filaments is confined exclusively to the lamellipodium and specifically to sites at its front edge. As Theriot and Mitchison (1991) have shown, the actin filaments of the central lamellipodium of the keratocyte do not move relative to the substratum. Therefore, the rate of protrusion at the cell front is the same as the rate of filament growth. As a consequence of their diagonal organization (Small et al., 1995), the actin filaments will translate laterally along the cell edge as the cell moves. Continuous nucleation of new filaments at the front edge of the lamellipodium will serve to maintain a constant filament density and to feed this filament flow. In the course of the cell's forward translocation, filaments will collect in the flanks of the lamellipodium and, we propose, become incorporated into the lateral axles of actin bundles. The data of Lee et al. (1993) and our own unpublished observations suggest that the cytoskeleton of the lateral flanks is subjected to inwardly directed contractile forces that retract actin filaments into the cell body. We surmise that the traction filaments first populate the base of the cell body. As the cell body rolls, filaments at the base rotate up and over it from behind and are continually replaced by new filaments supplied via the actin bundles. In this way, a cortical cage of
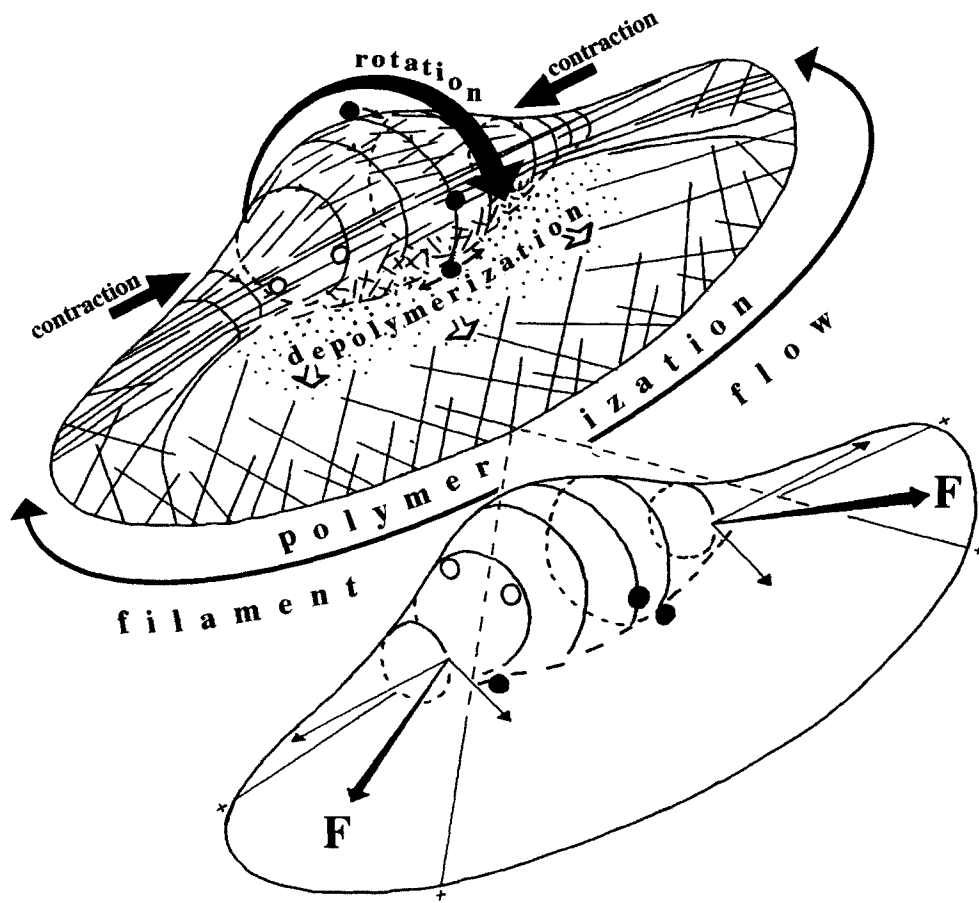
Figure 10. Proposed model of actin filament dynamics and cell movement in the kerato-cyte. Actin filaments are nu-cleated and polymerize at the front edge of the lamellipo-dium. Owing to their diagonal orientation, their growth leads to their displacement laterally as the cell moves (*dotted lines*), giving rise to a filament flow towards the lateral flanks of the lamellipodium. Filaments that reach the lateral flanks form bundles at the ends of the cell body and are retracted into the cell body cortex. The interaction of myosin and actin around the cell body leads to tension development, which maintains cell body shape, and to a component of force (F) that drives the translocation of the cell body, involving its ro-tation. Force diagram indicates that the lateral components of F cancel each other, and a net forward component remains. At the base of the lamellipo-dium, the depolymerization of the trailing ends of lamellipo-dium filaments and of fila-ments within the cell body re-plenishes the actin monomer pool. (*Open and closed cir-cles*) Beads that remain on the cell surface (*closed circles*) or are endocytosed (*open circles*). For other details, see text.

actin filaments could be created, which, coupled with myo-sin, would constrain the cell body in its elongated form. Translocation of the cell body is presumed to result from a net component of the myosin-based force in the direction of locomotion that arises from the net forward orientation of filaments emanating from the spindle ends of the cell body into the lateral lamellipodium (Fig. 10). Thus, as in other cells, myosin is attributed a role in cell body translo-cation (Chen, 1981; Fukui et al., 1989) involving the break-ing of trailing contacts with the substrate (Small, 1989; Jay et al., 1995). For the keratocyte, there is a subtle variation on this theme, involving a rolling motion of the major part of the cell.

In this scheme, we must also consider how the actin fila-ments are recycled. One possibility is that the boundary between the front of the cell body and the lamellipodium serves as a sink where actin filaments of the cortical cage are disassembled. The observation that applied Con A–coated beads, which are presumably linked to actin filaments in the cell body via transmembrane complexes, moved side-ways, independently of the cell body, when they reached the border with the lamellipodium suggests that this bor-der may correspond to a region of disassembly. Juxta-posed with this boundary, at the rear of the lamellipodium, actin filament concentration is at a minimum (Small et al.,

1995), indicating that the concentration of factors favoring filament breakdown and monomer formation is probably high. By this route, the pool of G-actin could readily be re-plenished to feed lamellipodium protrusion and to main-tain the actin filament cycle.

Another interesting aspect of the rolling motion con-cerns the turnover of membrane components. Former studies that used gold particle probes to track membrane movements (Kucik et al., 1990, 1991) indicated that the membrane of the keratocyte lamellipodium moves pas-sively forward with the leading front. The rotating cell body puts this membrane movement in another perspec-tive. Activated beads that were bombarded onto the rear of the cell and stuck to its outer surface moved over the cell body to its front border with the lamellipodium, indi-cating that membrane components from the ventral sur-face were returned again to the front dorsal surface via this route. Membrane cascading into the base of the lamel-lipodium could thus serve to replenish membrane compo-nents drawn forward by protrusion.

Can we learn anything from the keratocyte about the dynamics of actin filaments and motility in general? While we shall refrain from making detailed speculations, it is our contention that the lamellipodium may turn out to be the primary site of actin filament nucleation in motile cells.

By means of filament flow and translocation, the lamellipodium could, in fibroblasts, for example, deliver filaments required for the initiation of stress fibers and the formation of cortical arcs, which both find their origin at the base of lamellipodia (Heath and Holifield, 1993; Small et al., 1996). To what extent cell body rolling is a feature of other fast-moving cells is also a matter worthy of future attention.

Note Added in Proof. The full-length sequences used to generate the figures are available via the World Wide Web at http://server1.imolbio.oeaw.ac.at.

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