Comparison of Actin and Cell Surface Dynamics in Motile Fibroblasts

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Abstract. We have investigated the dynamic behavior of actin in fibroblast lamellipodia using photoactivation of fluorescence. Activated regions of caged resorufin (CR)-labeled actin in lamellipodia of IMR 90 and MC7 3T3 fibroblasts were observed to move centripetally over time. Thus in these cells, actin filaments move centripetally relative to the substrate. Rates were characteristic for each cell type; 0.66 ± 0.27 μm/min in IMR 90 and 0.36 ± 0.16 μm/min in MC7 3T3 cells. In neither case was there any correlation between the rate of actin movement and the rate of lamellipodial protrusion. The half-life of the activated CR-actin filaments was ~1 min in IMR 90 lamellipodia, and ~3 min in MC7 3T3 lamellipodia. Thus continuous filament turnover accompanies centripetal movement. In both cell types, the length of time required for a section of the actin meshwork to traverse the lamellipodium was several times longer than the filament half-life. The dynamic behavior of the dorsal surface of the cell was also observed by tracking lectin-coated beads on the surface and phase-dense features within lamellipodia of MC7 3T3 cells. The movement of these dorsal features occurred at rates approximately three times faster than the rate of movement of the underlying bulk actin cytoskeleton, even when measured in the same individual cells. Thus the transport of these dorsal features must occur by some mechanism other than simple attachment to the moving bulk actin cytoskeleton.

FIBROBLASTS have been used as a model system for the study of cell motility for several decades. An important structure for motility is the leading edge, where filopodia and lamellipodia protrude forwards. The lamellipodium of motile fibroblasts is a highly dynamic, actin-rich structure 3–10 μm wide, which can rapidly protrude and retract. In addition, several different features including particles on the dorsal surface of the cell (Abercrombie et al., 1970*b*; Fisher et al., 1988), patches of membrane proteins (Holifield and Jacobson, 1991), actin (Wang, 1985; Okabe and Hirokawa, 1991), and actin-rich ruffles (Abercrombie et al., 1970*a*) continuously move centripetally from the leading edge toward the cell body in lamellipodia. This striking flow of cortical structures is observed only in motile cell types and is assumed to reflect behavior necessary for locomotion (Harris, 1973).

Several recent lines of experiment have directly demonstrated that actin and actin-containing structures move centripetally in the lamellipodium. Wang (1985) demonstrated that a spot photobleached on a fibroblast lamellipodium containing fluorescent actin polymer would move towards the cell body, at a rate of 0.8 μm/min. Similar photobleaching experiments in neuron growth cones have given a similar result; in these cells, actin polymer in the lamellipodium moves centripetally at a maximum rate of ~1.5 μm/min, although centripetal movement is only observed in a subset of cells (Okabe and Hirokawa, 1991). These results, along with preferential localization of newly incorporated microinjected actin monomer to the extreme leading edge of the lamellipodium (Okabe and Hirokawa, 1989, 1991; Symons and Mitchison, 1991) have given rise to the view that actin polymer in lamellipodia is formed primarily at the leading edge, and that the polymer is then slowly transported centripetally.

Features on the dorsal surface of the fibroblast lamellipodium including actin-rich ruffles (nonadherent lamellipodia lifted onto dorsal surface; Abercrombie et al., 1970a), patches of cross-linked membrane proteins (Holifield and Jacobson, 1991), and beads external to the lamellipodium (Abercrombie et al., 1970*b*; Fisher et al., 1988) as well as less clearly defined vesicles and cytoplasmic densities inside the cell (Fisher et al., 1988) also have been directly observed to move centripetally, using DIC, phase-contrast, or fluorescence videomicroscopy. Since external markers such as beads and patches move at the same rate as internal markers such as vesicles and actin bundles (Fisher et al., 1988; Holifield and Jacobson, 1991), it has been widely postulated that these sorts of dorsal movements all represent passive attachment to the underlying, moving actin cytoskeleton. Important support for this idea comes from work in *Aplysia* bag cell growth cones, where the rate of movement of beads on the dorsal surface of the cell is identical to the rate of centripetal movement of the actin meshwork after treatment with cytochalasin (Forscher and Smith, 1988, 1990). However, in fibroblasts, the rate of movement of bulk actin poly-

mer determined by photobleaching (0.8 μm/min; Wang, 1985) is substantially slower than typical rates of movement reported for dorsal structures (up to 15 μm/min; Fisher et al., 1988). It has not been clear whether this rate disparity is simply reflective of differences among different fibroblast types, or is symptomatic of the presence of two distinct mechanisms giving rise to centripetal flow in the lamellipodium of actin and dorsal structures, respectively, at different rates.

In none of the previously reported studies on centripetal movement of actin or dorsal features in fibroblasts has the rate of centripetal transport been correlated with the rate of lamellipodial extension, so the relationship between centripetal flux and cell protrusion is unclear. We have previously described the dynamic behavior of actin filaments in lamellipodia of highly motile goldfish epithelial keratocytes (Theriot and Mitchison, 1991), using the recently developed technique of photoactivation of fluorescence (Mitchison, 1988). In keratocytes, the marked actin filaments in the lamellipodium remain stationary relative to the substrate as the cell moves forward over them, regardless of cell speed. This implies that in these cells, the rate of cell locomotion is directly correlated to the rate of insertion of new actin polymer at the leading edge. In fibroblasts, where the actin does not remain stationary but rather moves centripetally relative to the substrate, there may be a more complex relationship between the rate of actin movement and the rate of lamellipodial protrusion.

In this report we have used fluorescence photoactivation to mark and follow a spatially defined subset of actin filaments in the lamellipodia of motile fibroblasts. We have been able to observe both the spatial movements of actin filaments and the rate of filament turnover in lamellipodia, and correlate them with the rate of cell protrusion. In addition, we have directly tested the assumption that the movement of dorsal features and cytoplasmic waves reflects the movement of the internal actin meshwork of the lamellipodium by measuring the rates of both actin movement and movement of beads on the dorsal surface and inhomogeneities within the lamellipodium in a single cell type under uniform conditions, and even within the same individual cells. These experiments increase our understanding of the dynamic organization of actin filaments in the moving lamellipodium, and challenge existing models concerning dorsal surface movements.

Materials and Methods

Cell Culture and Microinjection

MC7 3T3 cells were grown in MEM-EBSS with 5% FCS, penicillin, and streptomycin. Before microinjection, they were plated on 25-mm round, acid-washed glass coverslips and allowed to become confluent. Monolayers were wounded by rinsing the coverslip in Hank's BSS, gently scraping the tip of a pair of sterile duck-billed forceps across the coverslip, rinsing again, and returning to fresh medium. Wounded cells were allowed to recover in the incubator for at least 1 h before injection. IMR 90 cells were grown in F-12 with 10% FCS, penicillin, and streptomycin. For microinjection, the cells were plated on acid-washed coverslips in medium with 2% FCS and allowed to spread for 1–3 h before observation. For observation and microinjection, cells were mounted in a temperature-controlled aluminum chamber held at 30–32°C.

Caged resorufin-actin (CR-actin)[1] was prepared and microinjected as previously described (Theriot and Mitchison, 1991). CR-actin was allowed to incorporate in injected cells for at least 30 min before photoactivation.

Preparation of Lectin-coated Beads

0.5-μm diameter Polybead Amino Microspheres (Polysciences, Warrington, PA) were suspended in PBS and treated with 8% glutaraldehyde overnight. After rinsing, the activated beads were resuspended in 20 mM concanavalin A in PBS and rotated at room temperature for 5 h. The coated beads were resuspended in 0.5 M ethanolamine in PBS and rotated at room temperature for 1 h, then rinsed and stored in 10 mg/ml BSA in PBS with 0.02% NaN_3.

Photoactivation and Data Collection

The photoactivation and recording apparatus were essentially as previously described (Mitchison, 1988). Paired phase and fluorescence images for photoactivation experiments were collected using an ISIT camera as previously described (Theriot and Mitchison, 1991). Phase images for particle-tracking experiments were collected using a Hamamatsu Newvicon camera. All images were collected using MaxVision (Datacube, Peabody, MA) and stored on optical disc (Panasonic, Secaucus, NJ). Image analysis was performed using Image 1 version 3.94 (Universal Imaging Corp., Media, PA). Rates of actin flux and cell protrusion were determined by fitting a straight line to each plot of position vs. time, with position measured at least every 30 s. Fluorescence intensity profiles were determined by averaging three adjacent lines for each profile.

Results

Distribution of Endogenous Actin and CR-Actin in Lamellipodia

To produce stable lamellipodia oriented in a predictable direction, we wounded monolayers of MC7 3T3 fibroblasts and allowed them to recover. Wound-healing cells formed large stable lamellipodia with a dense meshwork of actin filaments, which excluded organelles. To determine the distribution of filaments within the lamellipodium, we fixed the cells and labeled them with fluorescent phalloidin. The density of F-actin in lamellipodia of these cells was highest at the extreme leading edge and decreased somewhat over the lamellipodium (Fig. 1), in agreement with previous measurements (Symons and Mitchison, 1991). The density of actin filaments at the rear of the lamellipodium was an average of 60% of the density at the front of the lamellipodium (SD = 14%, n = 12). CR-actin microinjected into the cells incorporated into endogenous actin structures including stress fibers and lamellipodia within 30 min (not shown).

Centripetal Movement of Actin Filaments in Lamellipodia

To probe actin dynamics in lamellipodia, CR-actin was injected into wound-healing MC7 3T3 fibroblasts or spreading IMR 90 human fibroblasts and photoactivated in a narrow bar at the leading edge of the lamellipodium. In IMR 90 cells, the activated bar moved centripetally over time, toward the cell body. Fig. 2 shows an example of flux in the lamellipodium of an IMR 90 cell. *a* and *b* show phase images of an injected activated cell immediately after and 1.5 min after activation. *c* and *d* show resorufin fluorescence at the same time points. *e* and *f* show composite superimposed images of both phase and fluorescence at these two time points. *g* and *h* show tracings of the cell boundary and of the outline of the activated fluorescent bar. The activated bar of actin filaments has moved centripetally, away from the boundary of the cell. *i* shows fluorescence intensity profiles of the activated bar at the early and later time points as thick and thin

1. *Abbreviation used in this paper*: CR-actin, caged resorufin actin.

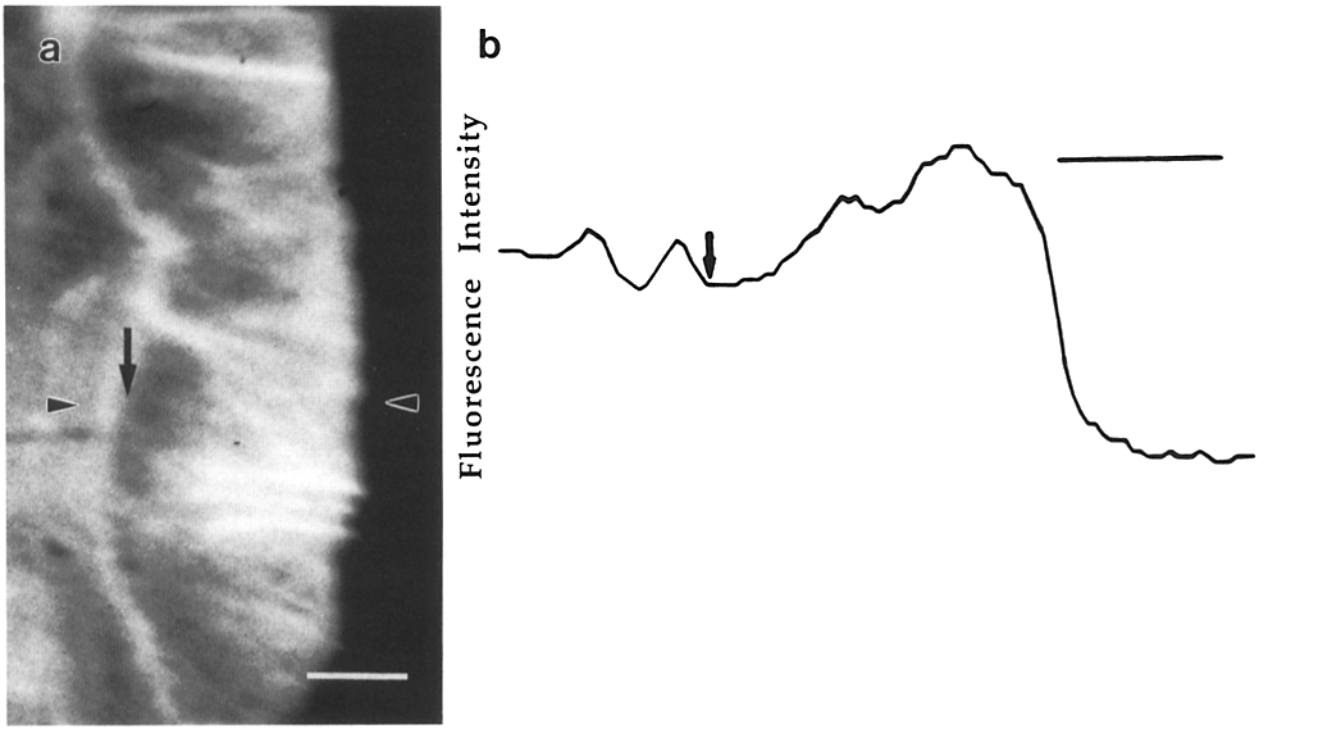

Figure 1. Actin filament density gradient in lamellipodia. (*a*) Lamellipodium of a wound-healing MC7 3T3 fibroblast labeled with fluorescent phalloidin. Arrowheads mark site of fluorescence intensity profile. (*b*) Fluorescence intensity profile through lamellipodium of cell shown in *a*. Arrows mark rear of lamellipodium in both panels. Bars, 5 μm.

lines, respectively. The activated bar has moved centripetally relative to the substrate as well as relative to the cell edge, and has decreased in intensity over time. The average rate of centripetal transport of actin in IMR 90 cells at 30–32°C was 0.66 μm/min (SD = 0.27, n = 10). Splitting of the bar was never observed; all the filaments in the bar appeared to behave as a single population.

Actin dynamics in lamellipodia were also examined in MC7 3T3 cells undergoing wound healing. Just as in IMR 90 fibroblasts, the activated actin bar in the lamellipodium of an injected MC7 3T3 cell moved centripetally toward the cell body. Fig. 3 shows an example of centripetal flux in an MC7 3T3 cell. The paired images were acquired immediately after (*a, c, e,* and *g*) and 4 min after (*b, d, f,* and *h*) activation. Fluorescence intensity profiles (*i*) reveal that in MC7 3T3 cells as well as IMR 90 cells, the activated actin bar moves centripetally relative to the substrate as well as relative to the cell margin, while decreasing in intensity over time. The average rate of centripetal transport of actin in MC7 3T3 cells at 30–32°C was 0.36 μm/min (SD = 0.16, n = 32). As in IMR 90 cells, bar splitting was never observed.

To determine the relationship between the rate of actin flux and the rate of lamellipodial protrusion, we measured the rate of movement of the leading edge of the cells in which we had examined centripetal actin transport. The rate of protrusion of the leading edge in the activated IMR 90 cells varied from 1.39 to −0.40 μm/min (slow retraction). There was no correlation between the rate of centripetal actin transport and the rate of cell protrusion (Fig. 4 *a*). In activated MC7 3T3 cells, the rate of lamellipodial protrusion varied from 0.48 to −0.23 μm/min. As was the case with IMR 90 fibroblasts, there was no correlation between the rate of actin movement and the rate of protrusion in MC7 3T3 cells, although there was considerable scatter in the actin transport rates (Fig. 4 *b*).

Compression of Actin Mesh Accompanying Centripetal Transport

We noted that activated bars of CR-actin in MC7 3T3 lamellipodia tended to narrow over time as the actin was transported centripetally (see, for example, Fig. 3 above, compare *c* and *d*). To determine whether this narrowing represented a compression of the meshwork or nonuniform loss of activated filaments in the bar, we followed the behavior of relatively broad bars covering most of the lamellipodium. In four cells activated with broad bars (3–4 μm across), the narrowing was accompanied by an increase in peak fluorescence intensity. Fig. 5 shows an MC7 3T3 cell immediately after (*a, c, e,* and *g*) and 3.5 min after (*b, d, f,* and *h*) activation of a bar which covers nearly all of the lamellipodium. The fluorescence intensity profiles (*i*) indicate that at the later time point, the bar has decreased in width by about 50%, while the peak fluorescence intensity has increased. This peak increase (which accompanies an actual decrease in the total fluorescence intensity in the bar) would not be observed if the narrowing were due solely to nonuniform filament turnover. Thus the actin meshwork in the lamellipodium is compressed over time, a behavior superimposed on transport of the entire meshwork toward the cell body. The compression could result from contraction of the meshwork or from reorientation of filaments in the meshwork.

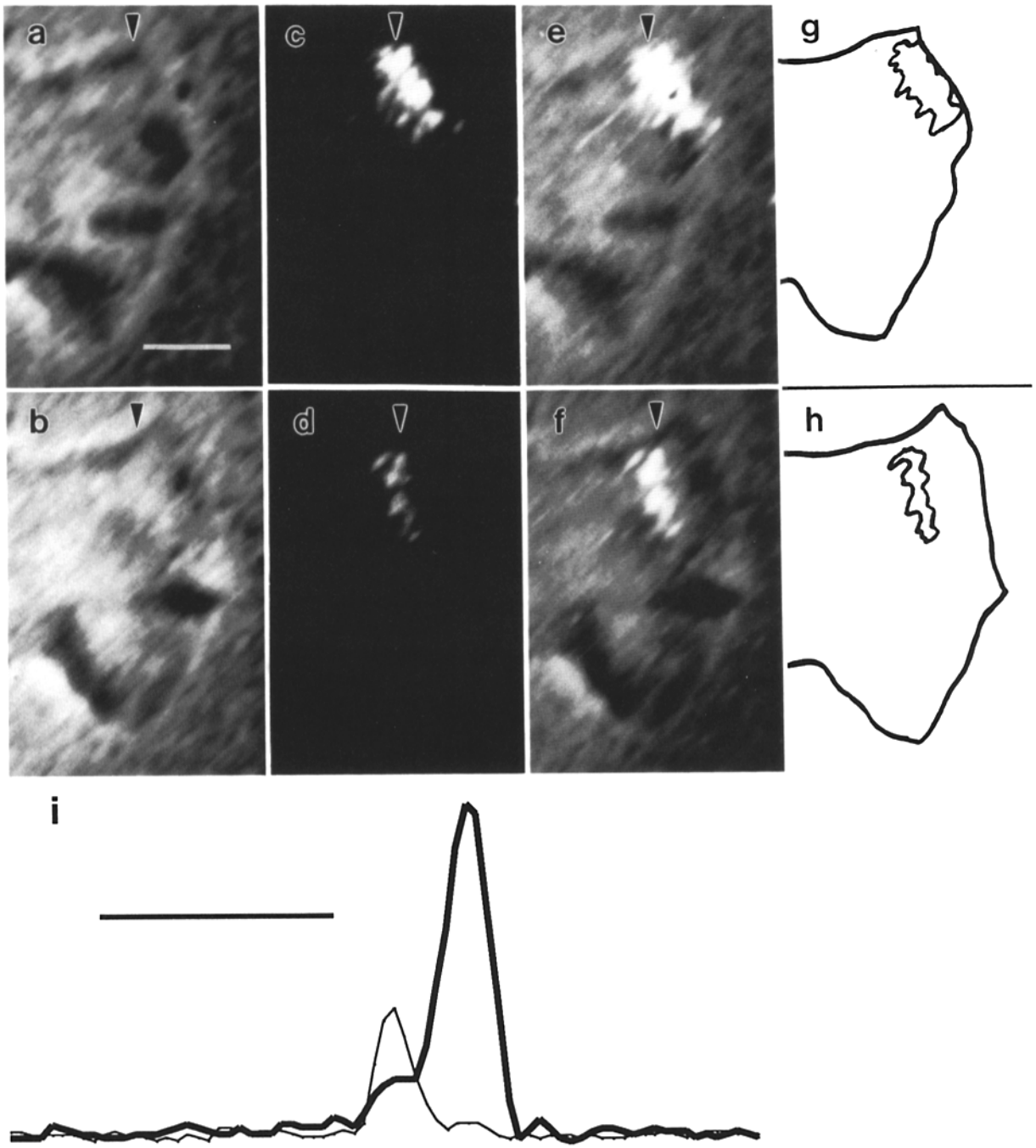

Figure 2. Movement of actin in IMR 90 fibroblast lamellipodia. (*a–f*) Paired phase (*a* and *b*) and fluorescence micrographs (*c* and *d*) and composite superimposed images of both phase and fluorescence (*e* and *f*) of lamellipodium 4 s (*a, c, e*) and 94 s (*b, d, f*) after activation. The arrowheads mark a fixed point in all panels. Bar, 5 μm. (*g* and *h*) Tracings of cell outline and outline of activated bar 4 s (*g*) and 94 s (*h*) after activation. (*i*) Fluorescence intensity profiles through this lamellipodium, thick line, 4 s, and thin line, 94 s, after activation. Cell is advancing from left to right. The poor quality of the phase image results from the use of the ISIT camera. Bars, 5 μm.

Rate of Turnover of Actin Filaments in Lamellipodia

The activated CR-actin bar in lamellipodia of both fibroblast cell lines lost fluorescence intensity over time. We assume that this represented disassembly of activated filaments and loss of disassembled activated monomer by diffusion because photobleaching was negligible under our observation conditions (Theriot and Mitchison, 1991). It was not possible to determine whether the diffusing species was truly individual monomers or short oligomers formed during depolymerization. To determine the average half-life of actin filaments in the activated region, we fit an exponential decay curve to each plot of total integrated fluorescence intensity vs. time (Fig. 6). In IMR 90 cells, the average half-life of filaments in the lamellipodium was 55 s (SD = 28, n = 10). In MC7 3T3 cells, the average half-life was 181 s (SD = 99,

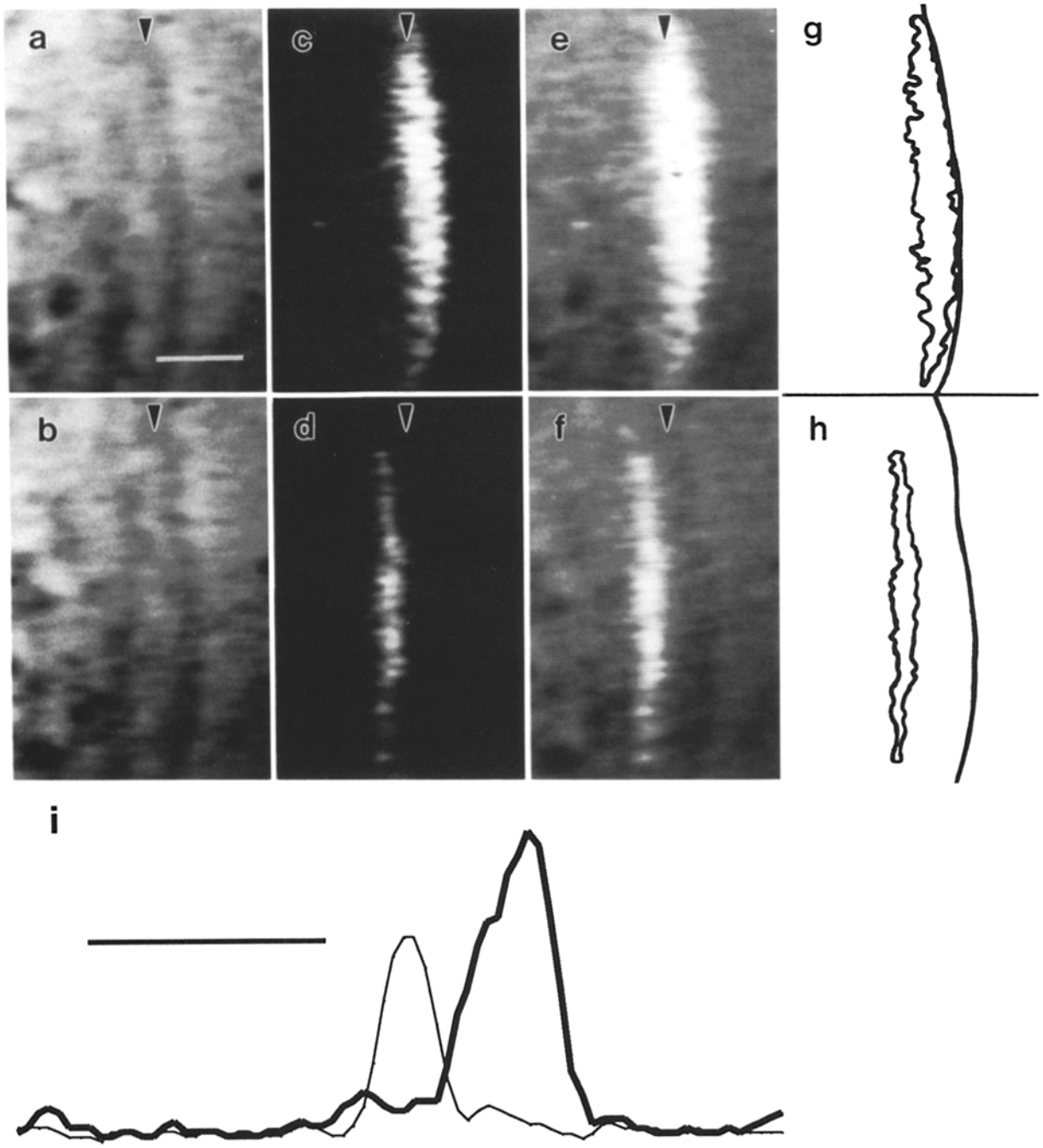

Figure 3. Movement of actin in MC7 3T3 fibroblast lamellipodia. (*a–f*) Paired phase (*a* and *b*) and fluorescence micrographs (*c* and *d*) and composite superimposed images of both phase and fluorescence (*e* and *f*) of lamellipodium 4 s (*a*, *c*, *e*) and 4 min (*b*, *d*, *f*) after activation. The arrowheads mark a fixed point in all panels. (*g* and *h*) Tracings of cell outline and outline of activated bar 4 s (*g*) and 4 min (*h*) after activation. (*i*) Fluorescence intensity profiles through this lamellipodium, thick line, 4 s, and thin line, 4 min, after activation. Bar, 5 μm.

n = 30). In neither case was there a strong correlation between filament half-life and rate of actin movement, or between filament half-life and rate of lamellipodial protrusion.

The lability of the actin filaments in the lamellipodium suggested that the entire lamellipodium might be dynamic; that is, that filaments might be polymerizing as well as depolymerizing over the entire structure. To determine whether polymerization throughout the lamellipodium were necessary to maintain the steady-state distribution of actin observed, we calculated the length of time it would take an average filament created at the leading edge to traverse the lamellipodium, and compared this time to the average filament half-life. The average width of the lamellipodia in the observed cells was 7.3 μm for IMR 90 cells (SD = 2.8, n = 10) and 5.5 μm for MC7 3T3 cells (SD = 1.3, n = 32). The average time the activated bar required to cross the lamellipodium was determined by dividing the width by the rate of actin movement with respect to the edge of the cell

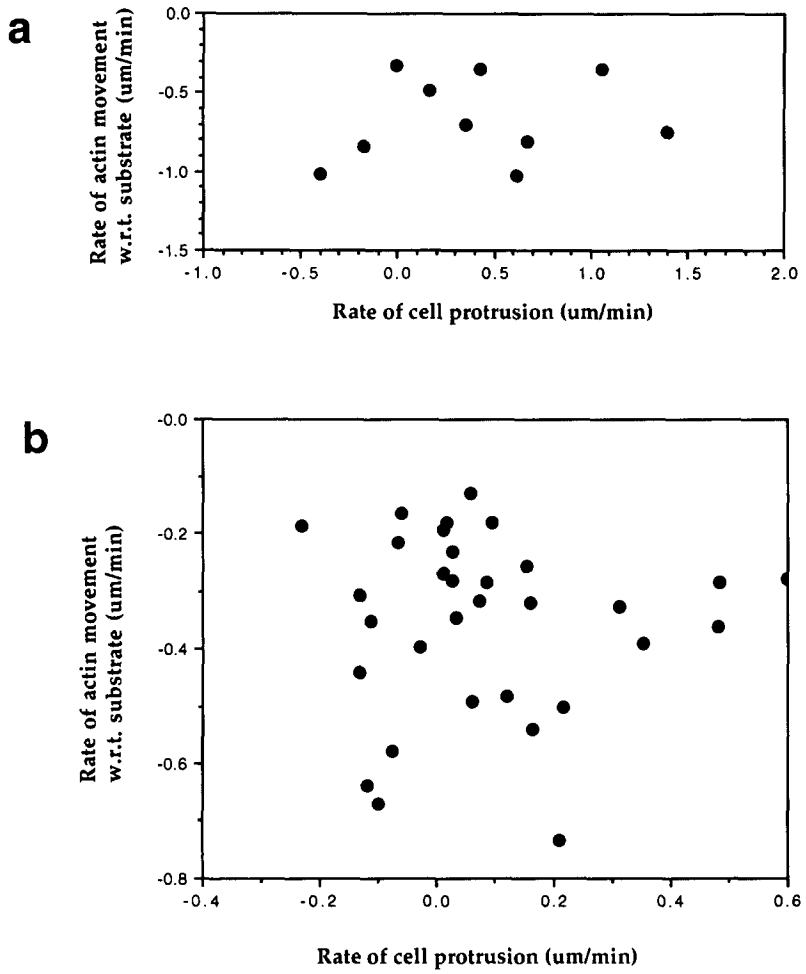

Figure 4. Correlation between rate of actin movement and rate of lateral cell protrusion, measured relative to the substrate. (*a*) IMR 90 fibroblasts. (*b*) MC7 3T3 fibroblasts. The total amount of protrusion may be slightly underestimated for each cell, since protrusion can also occur normal to the substrate plane.

for each individual cell. The average times to cross the lamellipodium in the observed cells were 7.6 min for IMR 90 cells (SD = 3.8, n = 10) and 15.6 min for MC7 3T3 cells (SD = 8.2, n = 32). This time can be expressed as a multiple of the actin filament half-life. In IMR 90 cells, actin requires approximately eight filament half-lives to cross the lamellipodium, and in MC7 3T3 cells, it requires approximately five filament half-lives. These data are summarized in Table I, and compared to the dynamic parameters of actin in goldfish epithelial keratocytes (Theriot and Mitchison, 1991).

Comparison of Actin Dynamics and Cell Surface Dynamics

The average rates reported for centripetal movements of particles on the cell surface of fibroblasts (Fisher et al., 1988) are typically faster than the rates found for centripetal movement of actin polymer (Wang, 1985; this paper). Without measuring both parameters in a single cell type, it is not possible to determine whether these differences are due merely to variations among different cells. To this end, we compared the rate of particle transport on the dorsal surface of lamellipodia of MC7 3T3 cells with the rate of centripetal actin movement in the same cell type. Movements on the dorsal surface of the fibroblast lamellipodia were tracked using 0.5-μm latex beads coated with Con A (Fig. 7). On wound-healing MC7 3T3 cells at 30–32°C, the beads were transported centripetally at an average rate of 1.12 μm/min (SD = 0.66, n = 18). This rate was significantly different from the rate of actin movement in this cell type under the same conditions, 0.36 μm/min (unpaired t-test, $P < 0.001$).

To test whether the difference in rates was an artifact due to damage to injected cells, we compared the rate of actin transport to the rate of movement of phase-dense cytoplasmic structures in the lamellipodia of the same individual injected cells. The rate of movement of these cytoplasmic structures is identical to the rate of bead transport in fibroblast lamellipodia (Fisher et al., 1988). Fig. 8 *a–c* show the movement of phase-dense inhomogeneities through the lamellipodium of an injected cell. Immediately after this recording, the cell was activated, and *d–l* show phase, fluorescence, and composite images of the same lamellipodium immediately after and 4 min after activation. For the injected cells where rates could be determined for both movement of cytoplasmic structures and actin, the average rate of movement of cytoplasmic densities was 0.95 μm/min (SD = 0.32, n = 13). This is not significantly different from the rate of dorsal bead transport measured above. The average rate of actin transport in this group of cells was 0.37 μm/min (SD = 0.12, n = 13), nearly identical to the average rate of actin transport for the total set of cells. The rates of actin transport and movement of phase-dense cytoplasmic phase-dense structures, here compared in the same individual cells, were significantly different (paired t-test, $P <$ 0.001). The result was the same whether the phase-dense particles were tracked before or after activation.

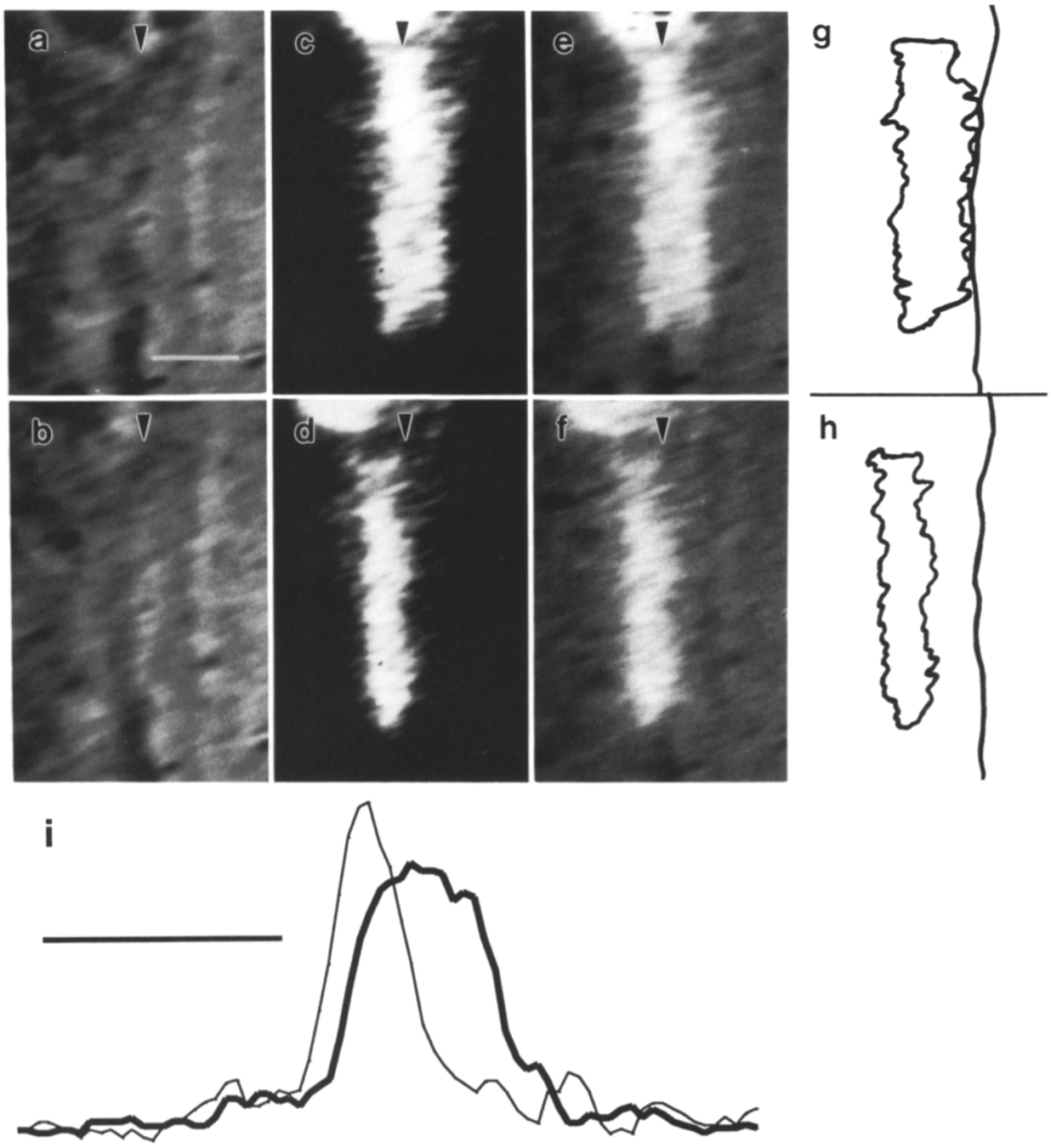

Figure 5. Activated bar narrows over time. (*a–f*) Paired phase (*a* and *b*) and fluorescence micrographs (*c* and *d*) and composite superimposed images of both phase and fluorescence (*e* and *f*) of lamellipodium 4 s (*a*, *c*, *e*) and 3.5 min (*b*, *d*, *f*) after activation of a wide bar, covering most of the lamellipodium. The arrowheads mark a fixed point in all panels. (*g* and *h*). Tracings of cell outline and outline of activated bar 4 s (*g*) and 3.5 min (*h*) after activation. (*i*) Fluorescence intensity profiles through this lamellipodium, thick line, 4 s, and thin line, 3.5 min after activation. Note the increase in absolute fluorescence intensity at the rear of the lamellipodium at the later time point. Bars, 5 μm.

Discussion

Centripetal Transport of Actin in Fibroblast Lamellipodia

Centripetal flow of cell structures, cross-linked patches of membrane proteins, and cell surface markers has been observed consistently in motile cells for decades (for a recent review see Heath and Holifield, 1991). This apparent flow was variously interpreted as centripetal movement of the cell membrane (Bretscher, 1976), contraction waves moving through the lamellipodium (Ambrose, 1961; Sorrano and Bell, 1982), and movement of actin polymer (Wang, 1985; Forscher and Smith, 1988; Fisher et al., 1988). Recent experiments have shown that the bulk plasma membrane does not flow centripetally in locomoting cells (Lee et al., 1990; Kucik et al., 1990; Holifield et al., 1990). Thus the ubiquitous centripetal flow observed in locomoting cells must be due to movements of actin polymer, contraction waves, or

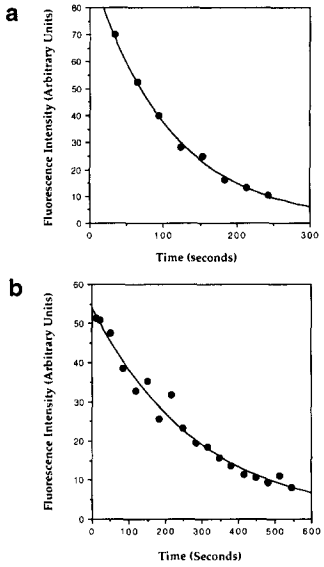

Figure 6. Fluorescence intensity decrease over time approximates exponential decay. (*a*) Plot of fluorescence intensity vs. time for an activated CR-actin bar in an IMR 90 lamellipodium. Solid line is best exponential fit. The average filament half-life for this cell was 68 s. (*b*) Plot of fluorescence intensity vs. time for an activated CR-actin bar in an MC7 3T3 lamellipodium. Solid line is best exponential fit. The average filament half-life for this cell was 199 s.

movements of an unidentified component of the lamellipodium.

Centripetal transport of actin (Wang, 1985; Okabe and Hirokawa, 1991) and actin-containing structures (Fisher et al., 1988; Svitkina et al., 1986) in fibroblast lamellipodia has been observed by several different methods, at rates ranging from 0.8 μm/min (Wang, 1985) to 15 μm/min (Fisher et al., 1988). Using the technique of photoactivation of fluorescence, we also observe centripetal transport of actin in two different mammalian fibroblast cell lines at rates similar to that measured previously by photobleaching of fluorescently labeled actin (Wang, 1985).

Photoactivation and photobleaching may in principle cause perturbation of the observed biological phenomena by damage to cellular structures by ultraviolet light. The 365-nm UV light used to photoactivate CR-actin is relatively harmless to biological structures (Hiramoto et al., 1984). Lower levels of light are used in photoactivation than in photobleaching, and photoactivation of microtubules has been shown to cause no breakage (Mitchison, 1988). Similarly, photobleaching of in vivo actin structures does not apparently perturb them (Wang, 1985). We have never observed any obvious behavioral or structural changes in living cells caused by photoactivation of either actin or tubulin, and the agreement between measurements of movement and turnover obtained using photoactivation and other methods gives us confidence that photoactivation does not seriously perturb the dynamic behavior of actin in lamellipodia.

Using methods other than photoactivation or photobleach-

Table I. Comparison of Dynamics in Fibroblast and Keratocyte Lamellipodia

	IMR 90	MC7 3T3	Keratocyte
Rate of actin movement w.r.t. substrate (μm/min)	−0.66 (0.27)	−0.36 (0.16)	−0.11 (0.70)
Rate of cell protrusion w.r.t. substrate (μm/min)	0.41 (0.55)	0.08 (0.20)	2.40 (1.74)
Rate of actin movement w.r.t. cell (μm/min)	−1.08 (0.57)	−0.44 (0.25)	−2.52 (1.75)
Actin half-life (s)	55 (28)	181 (99)	23 (6)
Width of lamellipodium (μm)	7.3 (2.8)	5.5 (1.3)	9.0 (2.1)
Time to cross lamellipodium (min)	7.6 (3.8)	15.6 (8.2)	3.8 (2.0)
Half-lives to cross lemellipodium	8	5	10

Comparison of dynamics in fibroblast and keratocyte lamellipodia. Standard deviations for each measurement are given in parentheses. Data for keratocytes are taken from Theriot and Mitchison (1991).

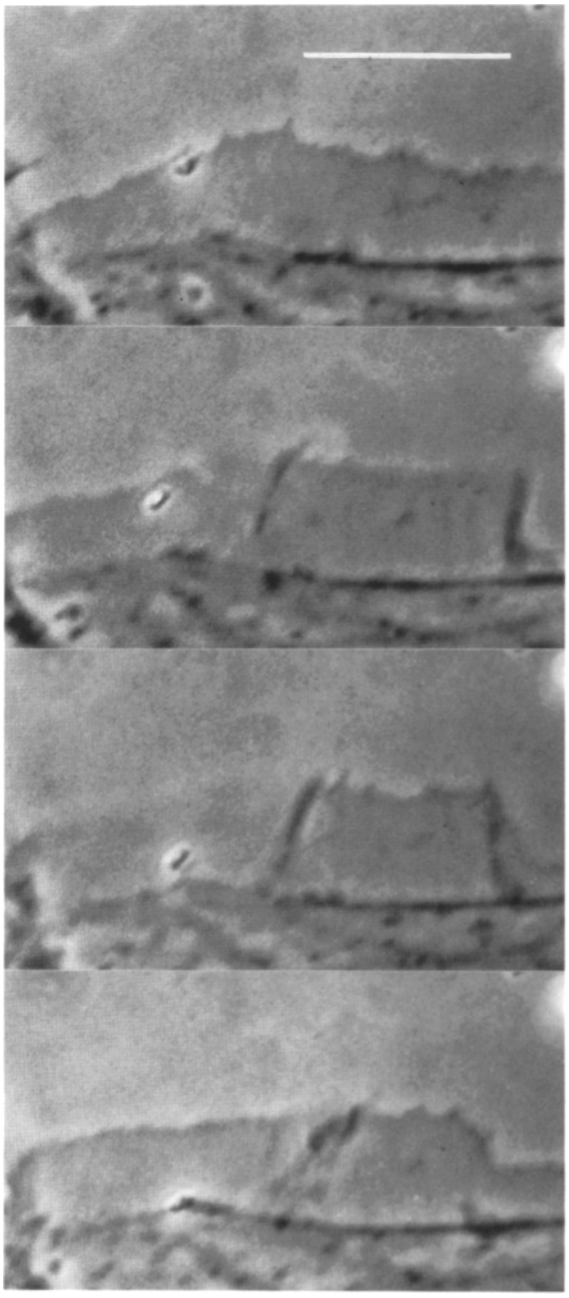

Figure 7. Lectin-coated bead transport on MC7 3T3 fibroblast lamellipodium. Frames taken at 30-s intervals. Bar, 10 μm.

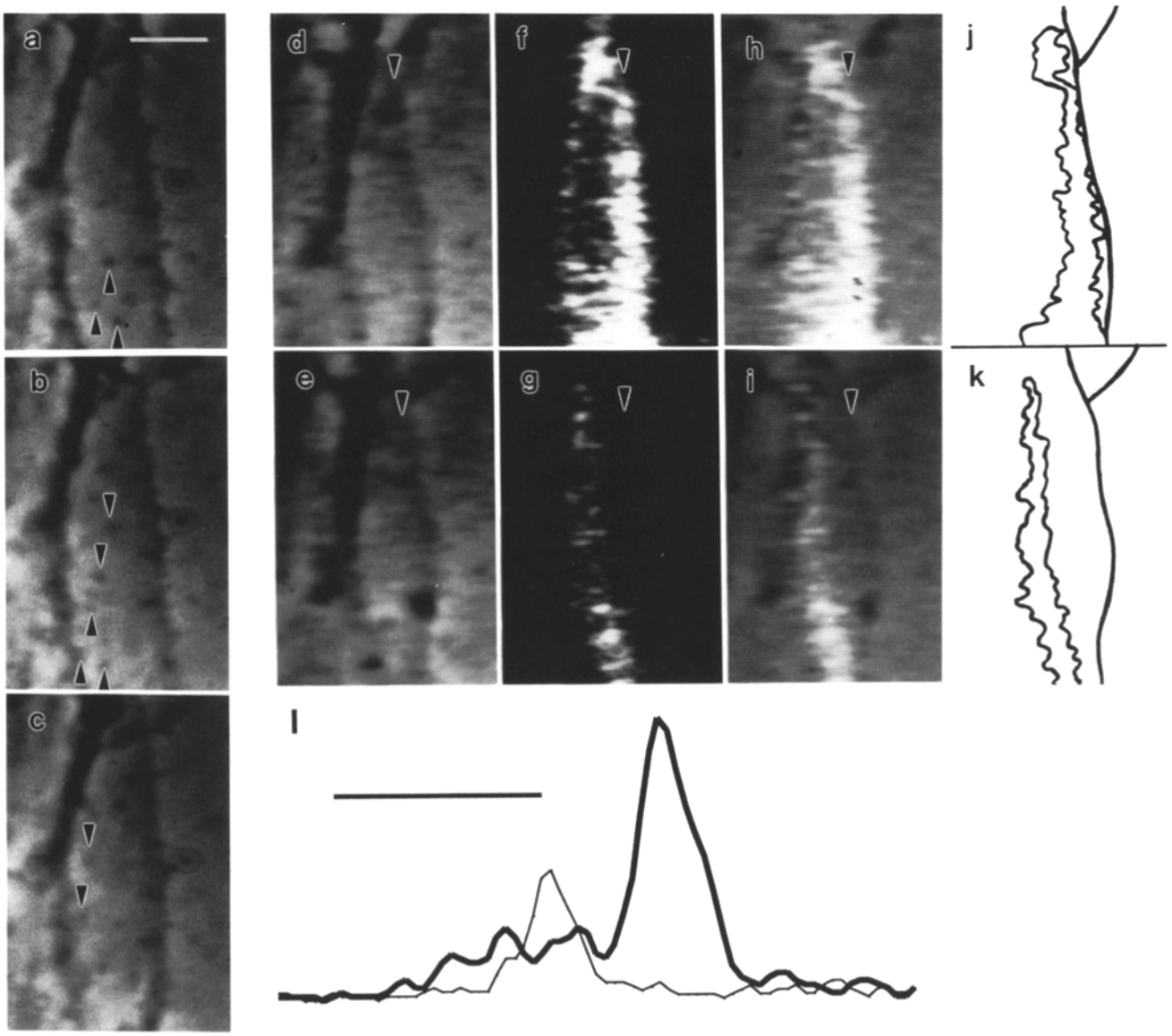

Figure 8. Comparison of movement of phase-dense inhomogeneities in lamellipodium with CR-actin in the same cell. (*a–c*) Movement of phase-dense particles through MC7 3T3 lamellipodium. Arrowheads mark small particles visible in sequential frames. Frames taken at 2-min intervals. (*d–i*) Paired phase (*d*, *e*), fluorescence (*f*, *g*) and composite superimposed phase and fluorescence (*h*, *i*) images showing movement of CR-actin in the same lamellipodium 4 s (*d*, *f*, *h*) and 4 min (*e*, *g*, *i*) after activation. The arrowheads mark a fixed point in *d–i*. (*j* and *k*) Tracings of cell outline and outline of activated bar 4 s (*j*) and 4 min (*k*) after activation. (*l*) Fluorescence intensity profiles through this lamellipodium, thick line, 4 s, and thin line, 4 min, after activation. Bars, 5 μm.

ing (typically phase or DIC videomicroscopy of moving cells, often coupled with immunofluorescence or electron microscopy), it has not been possible to determine how much of the actin in the lamellipodium is undergoing centripetal transport. We have determined that in both fibroblasts (this work) and goldfish epithelial keratocytes (Theriot and Mitchison, 1991), the actin in the lamellipodium appears to behave as a single coherent population, with no detectable shear among filaments. Given the limit of sensitivity of these photoactivation experiments, at least 90–95% of the actin in these lamellipodia behaves as if it is in this coherent population, either remaining essentially stationary relative to the substrate in keratocytes or moving centripetally slowly in fibroblasts. Thus the relative movement of actin away from the leading edge in locomoting cells appears not to be a behavior of only a subset of filaments in the lamellipodium, but is rather the behavior of a single, coherent meshwork throughout the structure. If our probe failed to incorporate into some subpopulation of filaments, we would then fail to observe the behavior of this subset. It seems unlikely that lamellipodia contain a subset of stable actin filaments because of their dynamic nature, and because they appear to quantitatively equilibrate with injected rhodamine-actin (Symons and Mitchison, 1991).

Correlation between Actin Transport and Protrusion

In none of the previously reported studies on centripetal movement of actin or actin-containing structures in fibroblasts has the rate of actin transport been correlated with the rate of lamellipodial extension, so it has not been clear what

role centripetal actin transport plays in cell locomotion. Our results indicate that the rate of actin flux is not correlated with the rate of protrusion, although the rate of rearward actin flux relative to the substrate did vary between the two different cell types. This lack of correlation would be expected if the control mechanisms governing the rate of protrusion operate solely at the leading edge, and the behavior of actin throughout the rest of the lamellipodium were relatively independent of the rate of new actin insertion at the leading edge. Thus we infer that the rate of lamellipodial extension in fibroblasts is determined solely by the rate of actin filament insertion at the leading edge.

The identity of the force responsible for lamellipodial protrusion is unknown. Likely candidates include force produced by molecular motors, such as myosin-I (Smith, 1988), force produced by ATP hydrolysis linked to actin polymerization (Hill and Kirschner, 1982), and osmotic and hydrostatic forces (Oster and Perelson, 1987). Molecular motors would be expected to regulate protrusive force via a clutch mechanism (Mitchison and Kirschner, 1988), but in fibroblasts our data suggests that there is no clutch operating, because the rate of flux is not slower in rapidly protruding cells. Thus we favor the idea that the protrusion of the lamellipodium at the leading edge is driven only by actin polymerization and/or osmotic and hydrostatic forces. Molecular motors may still be responsible for centripetal actin flux. Since centripetal flux with respect to the substrate is not necessary for locomotion (Theriot and Mitchison, 1991), and the rate of flux relative to the substrate in fibroblasts is independent of protrusion, the forces driving flux and protrusion are likely to be completely distinct.

Actin Filament Turnover in Fibroblast Lamellipodia

The half-life of actin filaments in lamellipodia of fibroblasts (Okabe and Hirokawa, 1989), macrophages (Rinnerthaler et al., 1991) and epithelial keratocytes (Theriot and Mitchison, 1991) has been estimated by various methods and found to be between 23 s (in keratocytes) and 1–5 min (in fibroblasts). Here we have determined the half-life of actin in fibroblast lamellipodia to be ~1 min in IMR 90 cells and 3 min in MC7 3T3 cells, in good agreement with previous measurements in lamellipodia of different fibroblast types. Comparing these cell lines and our previous study of keratocytes, we note the trend that the filament half-life is shorter in more rapidly moving cells.

In both types of fibroblasts studied as well as keratocytes, the time it would take for a single actin filament to travel (relative to the cell) the width of the lamellipodium is 5–10 times longer than the average half-life of the actin filaments (Table I). If actin filaments were polymerized only at the leading edge but depolymerized elsewhere, the relatively rapid turnover times of actin in the lamellipodium would predict a sharp gradient of filament distribution. For MC7 3T3 cells, for example, it would take five half-lives for a filament to cross the lamellipodium and only $1/2^5$ or ~3% of the filaments present at the leading edge should persist at the back of the lamellipodium. This is not the case; there is a shallow gradient of filament distribution in fibroblasts (Fig. 1), but the filament density falls on average only 40%. Thus there must be some actin polymerization throughout the lamellipodium as well as depolymerization, to maintain the steady-state distribution observed. Similarly, permeabilized cell experiments have indicated that actin nucleation sites are present throughout the fibroblast lamellipodium, but are about two to three times more dense at the leading edge than further back in the lamellipodium (Symons and Mitchison, 1991).

Disparity in Rates of Actin Movement and Particle Transport

It has previously been demonstrated (Fisher et al., 1988) that the rates of movement of beads on the surface of fibroblasts and of vesicular structures, small actin arcs, and other actin-containing structures within the lamellipodium are identical. In addition, the rate of transport of membrane protein patches is identical to the rate of movement of actin-containing arcs (Holifield and Jacobson, 1991). These observations along with many others gave rise to the assumption that dorsal structures on the cell moved centripetally by passive attachment to the internal actin cytoskeleton, which was also moving. However, by comparing the movements of the bulk of filamentous actin in the lamellipodium and both beads on the dorsal surface and phase-dense particles within the cytoplasm, we have directly demonstrated that this is not the case; the dorsal structures move several times more rapidly than the bulk actin meshwork. The centripetal movement of the actin meshwork observed in this work and in previous photobleaching experiments (Wang, 1985; Okabe and Hirokawa, 1991) cannot account for the ubiquitous centripetal flow of the cell surface structures seen in motile cells.

There are several viable hypotheses to explain the observed several-fold difference between the rate at which the bulk of filamentous actin moves in the lamellipodium and the rate at which particles move on the dorsal surface. First, particles on the dorsal surface of the cell may move by association with a rapidly moving structure which is distinct from the large bulk actin meshwork we observe by photoactivation. This second structure may be a contractile actin filament meshwork (Bray and White, 1988) which comprises only a small fraction of the total population of actin filaments in the lamellipodium, it may be an actin-fodrin meshwork, or it may be a nonactin structure. Second, molecular motors may actively transport beads over the lamellipodium. Third, it is possible that the "cytoplasmic waves" observed by phase-contrast and DIC microscopy in the lamellipodium do not represent transport of material, but rather compression waves (Ambrose, 1961; Sorrano and Bell, 1982; Byelintsev and Baranov, 1990). Beads on the dorsal surface and vesicles inside the cell might move at the faster rate of the compression wave using cycles of attachment and detachment coordinated with contraction and relaxation of the meshwork (Dembo and Harris, 1981).

In contrast to our results in fibroblasts and keratocytes, the study on immobilized *Aplysia* growth cones (Forscher and Smith, 1988) seems to indicate that the rate of centripetal actin movement is the same as the rate of dorsal structure transport. When cytochalasin was added to strongly adherent growth cones to prevent further polymerization of actin filaments, the meshwork inside the lamellipodium was observed to detach from the leading edge and move centripetally at the same rate at which beads (Forscher and Smith, 1990) and internal structures had previously been observed to move. Furthermore, when the cytochalasin was removed, the

meshwork was observed to regrow from the leading edge, filling the lamellipodium again at the same rate (Forscher and Smith, 1988). We are at present unable to account for the marked difference in our results. We consider three possibilities. (*a*) The behavior in growth cones is qualitatively different from the behavior in fibroblasts and keratocytes. (*b*) The addition of cytochalasin perturbed the structure of the growth cone in such a way as to cause the bulk meshwork to be transported at the faster, dorsal, rate. The transport mechanisms of the cell may recognize a detached, nondynamic lamellipodial meshwork as an arc or ruffle, and transport it accordingly. (*c*) The rate of movement of the actin meshwork after addition of cytochalasin is determined by the inherent contractility of the cell cytoplasm, and is fortuitously identical to the transport rate.

General Conclusions

Comparing the present study on fibroblasts with our previous work on epithelial keratocytes (Theriot and Mitchison, 1991), it is evident that these motile cell types share important dynamic behaviors in the lamellipodium. First, in both cell types, the rate of cell protrusion is not correlated with the rate of centripetal actin flux. This is suggestive of the possibility that the rate of movement of these cells is mechanistically determined by control mechanisms regulating the rate of actin polymer formation. It also suggests that the cell may use actin polymerization directly as a means of generating protrusive force. Second, in both fibroblasts and keratocytes the actin filaments are dynamic (continuously polymerizing and depolymerizing) throughout the structure. This implies that new actin filaments formed at the leading edge are subsequently released and incorporated into the crosslinked actin gel of the lamellipodium, which continues its typical constant dynamic behavior regardless of the rate of new polymer insertion. Thus the mechanisms controlling rate of actin polymer formation at the leading edge and controlling rates of depolymerization and polymerization throughout the rest of the lamellipodium must to some extent be independently regulated. Third, the rearward transport of dorsal features almost universally observed in motile cells is not, at least in fibroblasts, a direct reflection of the movement of the bulk actin meshwork inside the lamellipodium. Bulk actin filament transport and dorsal feature transport either must occur by independent mechanisms, or else must be only weakly coupled to each other. Our studies strongly support the view that the basic mechanisms of lamellipodial protrusion and associated actin dynamics are similar in different motile cell types, with quantitative variations in the relevant dynamic parameters, but qualitatively identical behavior.

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