Distribution of Actin in Spreading Macrophages: A Comparative Study on Living and Fixed Cells

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ABSTRACT The distribution of actin in proteose peptone-elicited murine peritoneal macrophages is examined with fluorescent analog cytochemistry (FAC), immunofluorescence, and electron microscopy (EM). Living adherent macrophages, microinjected with 5-iodoacetamido fluorescein-labeled actin, show a rather uniform distribution of actin with punctate and linear fluorescence in the thin peripheral areas of the cell. Apparent incorporation of a portion of the microinjected actin into the cell's actin cytoskeleton is also demonstrated when microinjected cells are subsequently examined for fluorescein fluorescence after fixation and extraction. However, a substantial perinuclear pool of actin, observed with FAC, is lost when microinjected cells are prepared for immunofluorescence using standard fixation methods. These results suggest that part of the cellular actin, possibly nonfilamentous or oligomeric, can be extracted during the normal preparative steps for immunofluorescence. When the dynamic distribution of actin structures is examined in living cells, extension of the cell's periphery is associated with the formation of punctate structures. The distribution of the most stable, nonextractable actin structures in fixed cells at different stages of spreading is quantified using rhodamine-labeled phalloidin and antiactin indirect immunofluorescence. At early stages, the rounded cells show cortical bands of fluorescence surrounding the nuclear region with punctate structures directly above the plane of the attached plasma membrane. At later time periods, fully spread cells contain both punctate and linear fluorescent structures. Adherent macrophage membranes, a preparation in which the attached membrane and membrane-cortex are isolated by shearing away the unattached plasma membrane and underlying cytoplasrn, show punctate and linear fluorescence when stained with rhodamine-labeled phalloidin. When the same cell remnant is negatively stained and examined with EM, the fluorescent punctate structures coincide with electron-dense foci and associated radiating thin filaments. We suggest that the optimal approach for elucidating the distribution of cytoskeletal and contractile proteins involved in motile processes is a combined approach using all three techniques. Although each technique is subject to potential artifacts and limitations, the use of FAC can permit the visualization of both the soluble and stabilized components of the cytoskeleton in living, functional cells. A qualitative method for determining differences in local concentrations of proteins is also presented.

The spreading of macrophages on a substrate apparently involves changes in the distribution of several contractile and cytoskeletal proteins as revealed by immunofluorescence (4, 15, 17, 25, 33) and electron microscopy (1, 20). However, the exact localization and organization of the major contractile and cytoskeletal proteins, including actin, has yet to be determined in vivo. Immunofluorescence and electron microscopy, although valuable tools, are limited since the fixation and
by applying back-pressure to needles secured in a Leitz electrode holder. Macrophages are microinjected in RPMI without Phenol red at 37°C on a temperature-regulated microscope stage (Cambion Cambridge Thermionic Corp., Cambridge, MA). In all cases the volume of injected solution is the minimum volume needed to produce detectable fluorescence using an image intensifier system (21, 26) and is measured to be approximately 5–10% of the total cell volume. It is calculated that the amount of microinjected is ~1.3 × 10⁻⁹ microliters and only ~4% of the total endogenous actin. Cell viability after microinjection is assessed by examining the morphology of injected cells with Nomarski optics (i.e., damaged cells display a loss of cytoplasmic structure and assume an inflated appearance). Viable cells also retain pre-injected morphology and exhibit phagocytosis of opsonized erythrocytes (Amato, P. A. and D. L. Taylor, manuscript in preparation).

For experiments in which microinjected cells are subsequently prepared for immunofluorescence, the position of the microinjected cell is noted with the stage verniers, as well as other characteristics such as cell shape, location of the nucleus, and lamellipodia. Immediately after recording the distribution of injected proteins in the living cell, the slide is rinsed and immersed in fixative. After staining, the injected cell is relocated and the fluorescent pattern is recorded with the video system and on 35mm film.

**Fixation and Staining of Whole Cells:** Cells to be stained with rhodamine-labeled phallolidin, a cyclic peptide that specifically binds to F-actin (3, 41), or with antiactin are washed twice in phosphate-buffered saline (PBS) and then fixed in 2–3.7% formaldehyde at 37°C for 30 min. After washing twice with PBS, cells are extracted with −20°C acetone for 3–5 min and air-dried. Rhodamine-labeled phallolidin (a gift of Dr. Thomas Wietand, Max Planck Institute for Medical Research) is stored in methanol at 0.2 mg/ml. For staining, 100 µl of 0.16 µg rhodamine-phallolidin/ml PBS is applied to the slides and incubated for 20 min at room temperature. Slides are rinsed twice with PBS and immediately viewed and photographed. NBD-phallacidin (a gift of Drs. Larry Barak and Walt Webb, Cornell University) is also used for some experiments. For indirect immunofluorescence, fixed and extracted cells are incubated with 70 µl of purified rabbit antiactin solution (0.05 mg/ml PBS; a gift of Dr. Keigi Fujiwara, Harvard Medical School) at 37°C for 45 min in a chamber of high humidity. After washing three times with PBS, cells are incubated with 70 µl of rhodamine-labeled goat anti-rabbit IgG (0.05 mg/ml PBS; N. L. Cappel Laboratories, Inc., Cochranville, PA) at 37°C for 30 min. After washing three times with PBS, cells are immediately viewed and photographed.

**Fixation and Staining of Adherent Membranes:** Macrophage adherent membranes are prepared essentially as described by Boyles and Bainton (5) and Clarke et al. (6). Macrophages are allowed to adhere and spread on London finder electron microscope grids and sheared in a stream of buffer (50 mM KCl, 2 mM MgCl₂, 2 mM EGTA, 10 mM PIPES, pH 6.7) from a syringe. The grids are immediately placed in a solution of 2% glutaraldehyde in the same buffer for 30 min. After rinsing, the grids are stained with rhodamine-labeled phallolidin in buffer (dilutions and concentrations the same as for whole cell staining) and incubated for 20 min. Grids are placed under a coverslip on a glass slide and photographed on the light microscope with a 63x Planapo oil immersion objective (N.A. = 1.4). They are then rinsed with distilled water, negatively stained with 1% uranyl acetate for 30 s at room temperature, blotted dry, and examined on the electron microscope.

**Microscopy:** A Zeiss photomicroscope equipped with epi-illumination fluorescence optics and Nomarski optics is used to view the cells. Microinjection is carried out under a 40x Achromatic water immersion objective (N.A. = 0.75) with a working distance of 1.6 mm. Examination of both microinjected cell fluorescence and fixed whole cell fluorescence is carried out with a 63x Plan-Neofluar water immersion objective (N.A. = 1.2). Fluorescent images of microinjected cells are recorded with a RCA TC 1030/H TV Silicon Intensified Target Camera coupled to an NEC ¾ inch cassette video tape recorder. An HBO 200-W mercury lamp (epi) or a 60-W tungsten lamp (substage) is the light source. Selected images on the video tape are photographed from the TV monitor (30) with a Nikkormat camera fitted with a 50 line/inch reticle grating (10) and Ilford HP5 film which is developed with Diafine developer. Cells treated with both rhodamine-labeled phallolidin and antiactin are photographed directly with the photomicroscope. For experiments in which microinjected cells are subsequently prepared for immunofluorescence, the position of the microinjected cell is noted with the stage verniers, as well as other characteristics such as cell shape, location of the nucleus, and lamellipodia. Immediately after recording the distribution of injected proteins in the living cell, the slide is rinsed and immersed in fixative. After staining, the injected cell is relocated and the fluorescent pattern is recorded with the video system and on 35mm film.

**RESULTS**

**Microinjection of Labeled Proteins**

Spread macrophages are microinjected with AF-actin, allowed to recover at 37°C, and examined with the fluorescence...
microscope. At 30 min postinjection, punctate and linear fluorescence is seen at thinner peripheral regions of the cell (Figs. 1b and d, and 2b; photographed from the TV monitor), suggesting rapid incorporation of labeled actin into the cytoskeleton. A large pool of injected actin is seen in the perinuclear region. The nucleus is delineated by a dark area of apparent exclusion of the labeled protein, indicating minimal nuclear-cytoplasmic exchange of labeled actin (Figs. 1d, 2b, and 3a). There is no increase in nuclear fluorescence of labeled actin for as long as 24 h postinjection (results not shown).

Fully spread cells are microinjected with labeled actin, allowed to recover for 30 min, and subsequently prepared for either rhodamine-phalloidin or antiactin staining to compare the results of these distinct methods. Fig. 2b, photographed from the TV monitor, shows a fully spread cell injected with labeled actin. The nucleus excludes labeled actin and the thin areas of cytoplasm show numerous punctate foci (Fig. 2b). Most importantly, after fixation, extraction, and staining with rhodamine-phalloidin, the same cell shows a large decrease of fluorescence in the perinuclear region, indicating a significant loss of labeled actin, presumably due to the extraction step required for immunofluorescent staining (Fig. 2d; photographed directly on the microscope with the automatic camera). In addition, punctate structures within the cell are more clearly observed. The differences in fluorescent images can be explained since Fig. 2b represents only the distribution of fluorescently labeled injected actin while Fig. 2d shows staining of total cellular actin (endogenous plus injected) remaining after fixation and extraction.

Fully spread cells microinjected with AF-actin are also examined for fluorescein fluorescence after fixation and extraction. Fig. 3 shows the fluorescence image of an AF-actin-injected cell both before and after fixation and extraction. The living cell (Fig. 3a; photographed from the TV monitor) shows dispersed fluorescence with punctate and linear structures in peripheral regions of the cell. After fixation and extraction (Fig. 3b; photographed from the TV monitor) the cell retains at least part of the actin in a “ghostlike” image, suggesting that at least a portion of the microinjected actin becomes incorporated into a stable actin cytoskeleton. The same cell, when stained with rhodamine-phalloidin and photographed from the TV monitor (Fig. 3c), shows intense staining of the stabilized pool of total cellular actin. When photographed directly with the camera in the microscope (Fig. 3d), distinct linear structures are seen in peripheral regions of the cell indicating a loss of some detail in the video image. The visualization of punctate and linear structures is more clearly observed when fluorescent images are photographed directly on the microscope (with an

![Figure 1](image-url)
 exposure time of 1–2 min) than when they are photographed from the video monitor (with exposure times of only a fraction of a second). The loss of image quality with the video image is offset by the ability to record living cells with a minimum of damage.

The distribution of actin structures during the spreading process is followed in the same cell (Fig. 4). After recording the initial actin distribution (Fig. 4b), the slides are placed in a petri dish containing complete culture media and the cells are allowed to undergo further spreading at 37°C and 5% CO₂. After 4 h, the cell becomes more flattened as shown in the Nomarski images (Fig. 4a and c); the nucleus is still delineated and remains in the same region of the cell (Fig. 4b and d). In the periphery, newly formed punctate structures are located in an area corresponding to flattened regions of the cell (Fig. 4c and d). There is no observable redistribution or concentration of actin to this leading edge. When fixed, extracted, and stained with rhodamine-labeled phalloidin (Fig. 4f; photographed from the TV monitor), the same cell shows a loss of fluorescence in the perinuclear area.

The distribution of actin is compared to that of ovalbumin, a control protein which is assumed to distribute evenly throughout the cytoplasm. Immediately after microinjection, labeled ovalbumin is apparently excluded from the nucleus (Fig. 5a). After 20 min, however, the nucleus becomes indistinct (Fig. 5b), suggesting that the labeled ovalbumin can diffuse slowly into the nucleus. When the ovalbumin-injected cell is stained with rhodamine-phalloidin (Fig. 5d and e), few punctate structures are seen. Immunofluorescent staining techniques produced a wide range in the number of fluorescent structures for each cell.

In cells microinjected with fluorescent molecules, local differences in fluorescence intensity can be due to differences in local concentration, pathlength of the fluorescent molecules, and/or the accessible volume in that region (29, 34, 36). In Fig. 5b, the thick perinuclear region and the thin cortical region both exhibit a relatively high fluorescence intensity. The high fluorescence intensity in the center of the cell could be due to a large pathlength. In addition, since the thin cortical region of cells usually excludes large organelles, the accessible volume for any small fluorescently labeled molecules should be greater in the cortex relative to other areas of the cell. The accessible volume for a fluorescent molecule in a particular region of a cell can be defined as total cytoplasmic volume minus organelle volume. Hence, local concentrations of labeled proteins cannot be directly inferred from fluorescence intensity alone. Some type of normalization method is required (34).

When compared to staining with rhodamine-labeled phalloidin, the distribution of labeled ovalbumin in the fixed and extracted cell shows a similar high fluorescence intensity in the thin, cortical regions of the cell (Fig. 5c [arrow] and d and e [arrow]). We can therefore conclude that there is no apparent
Figure 3  Fully spread macrophage microinjected with labeled actin, examined for fluorescein fluorescence after fixation and extraction, and subsequently stained with rhodamine-labeled phalloidin. a (TV monitor) shows the labeled actin distribution in the living cell with linear (arrow) and punctate structures in peripheral regions of the cell. The nucleus (N) apparently excludes labeled actin. b (TV monitor) shows fluorescein fluorescence after fixation and extraction; a portion of labeled actin is retained in the cell. c (TV monitor) and d (photographed on the photomicroscope) show the same cell stained with rhodamine-labeled phalloidin. Bar, 10 μm. × 1,600.

Characterization of the Stable Fraction of Actin during Spreading: A Quantitative Study

Rhodamine-phalloidin and antiactin antibodies are used to label the stable fraction of actin (fixed and maintained in the cell) during the spreading process. These methods are used to characterize the distribution of this pool of actin quantitatively. Cells are plated and allowed to spread for 15 min, 30 min, 1, 2, and 3 h. After fixation, extraction, and staining with rhodamine-labeled phalloidin, the slides are photographed and scored for both cell shape and the appearance of fluorescent structures. After 15 min of incubation, most of the cells (Fig. 6) are round (Fig. 7a) while a small percentage are beginning to spread. At a high plane of focus, a cortical band of fluorescence is seen (Fig. 7b); this is probably due to the distribution of actin structures surrounding the nucleus of the cell. At a low plane of focus (Fig. 7c), punctate structures are visible in the plane of substrate-membrane contact. After 30 min of spreading, a higher percentage of cells are spread with some cells assuming an elongated shape (Fig. 6); the cortical band of actin is seen at a high plane of focus (Fig. 8b) along with punctate structures in a low plane of focus (Fig. 8c). Linear fluorescent structures (referred to as fluorescent fibers in immunofluorescent studies) were only rarely seen after 30 min of spreading. However, after 1 h of spreading, linear fluorescence (i.e., fibers) is seen in all four categories of possible cell shapes (Fig. 6). After 3 h of incubation, most cells are spread with punctate and linear fluorescence dominating the images (Fig. 9a).

To compare indirect immunofluorescent images with those using rhodamine-labeled phalloidin, cells are allowed to spread for 15 min and for 3 h, and are subsequently fixed, extracted, and stained with either antiactin antibody or rhodamine-labeled phalloidin. Using the antibody, some cells exhibit only diffuse fluorescence at 15 min and no punctate structures are observed. After 3 h of incubation, many cells are fully spread and punctate structures can be seen above the attached membrane (Fig. 9b). Although linear fluorescent structures are observed using the antibody technique (Fig. 9b), they are seen less frequently and not as distinctly as they are when stained with rhodamine-labeled phalloidin. However, both techniques produced images which were nearly indistinguishable. A control experiment, in which cells were not exposed to the primary antibody but are incubated with rhodamine-labeled goat anti-rabbit IgG, shows no specific staining (not shown).
**Macrophage Adherent Membranes**

Adherent membranes are stained with rhodamine-labeled phalloidin, examined with the fluorescence microscope, then negatively stained and examined with the electron microscope in order to correlate fluorescent structures with ultrastructure in the same cell. Fluorescent images of adherent membranes resemble those of whole cells showing varying numbers of punctate structures as well as linear fluorescence (Fig. 10a and b). In the same cell, fluorescent structures coincide with electron dense areas at the ultrastructural level (Fig. 10c and d, and Fig. 11a and b). At high magnification, the electron dense foci are closely associated with meshworks of thin filaments (Fig. 11b) and may be similar to actin foci described for macrophages and other adherent cells using similar techniques (5, 14, 22, 23, 27, 31).
DISCUSSION

The Use of FAC to Study Actin Distribution

Electron and immunofluorescence microscopy have revealed that actin plays an important role in both cell adhesion and spreading (2, 9, 11, 13, 22, 23, 39). Reaven and Axline (20) demonstrated the presence of microfilaments on the cytoplasmic surface of the plasma membrane of macrophages. Scanning electron microscopy further revealed that the spreading lamellae and lamellipodia of macrophages are principally composed of dense foci, interconnected by radiating filaments and filament bundles, which may serve as elements linking actin to the substratum (31). Other studies, using actin antibodies to visualize actin-containing structures in macrophages, have similarly demonstrated the presence of fluorescent punctate structures which may serve as actin-substratum attachment sites (4, 16, 17). Although several studies using immunofluorescence have reported the absence of fluorescent actin fibers in these cells (15, 16, 18), Berlin and Oliver (4) have noted oriented fibers in fully spread macrophages. Hence, immunofluorescent and electron microscopic examination of macrophages has provided a good deal of information concerning the more stable structures of the cytoskeleton. However, conventional fixation and extraction procedures can result in the loss of cellular protein and even a reorganization of cellular components. Using various fixatives, Willingham and Yamada (40) report that as little as 50–70% of total cellular protein is retained in fixed cells under some conditions. Therefore, we applied the technique of flu-
the soluble actin pool in a living cell and stabilized actin variety of mechanisms (19, 38). It should be emphasized that injection volumes, allows for its use as a dynamic probe of both injected labeled actin is functionally active and is able to the injection of fluorescently labeled actin, especially in small within 30 min (7). The incorporation of labeled actin may result from the exchange of actin subunits in the cell by a variety of mechanisms (19, 38). It should be emphasized that the injection of fluorescently labeled actin, especially in small injection volumes, allows for its use as a dynamic probe of both the soluble actin pool in a living cell and stabilized actin structures. In addition, the visualization of distinct actin structures using FAC should not be expected to be as clearly detectable as with immunofluorescence since we are only looking at a small percentage of total actin.

Macrophages microinjected with labeled actin or labeled ovalbumin recover from the microinjection as shown by their normal morphology and by their ability to undergo spreading when placed in culture for as long as 24 h after injection (data not shown). Two lines of evidence suggest that the microinjected labeled actin is functionally active and is able to be incorporated into the endogenous contractile cytoskeleton: (a) the fluorescent actin forms punctate and linear structures in thin areas of the cytoplasm in the living cell, and (b) a fluorescent cytoskeletal "ghost" remains after fixation and extraction of cells which are previously injected with AF-actin. Previous studies (28, 30) have shown that labeled actin is functionally active in motile cell extracts and is able to incorporate into the endogenous actin pool of giant amoeba during amoeboid movement and various cellular processes. In this study, neither injected actin nor ovalbumin are sequestered into vesicles for at least 24 h, the maximum time injected macrophages have been maintained in culture.

**Comparison of FAC and Immunofluorescence Images: A Qualitative Method to Correct for Optical Artifacts**

Cells are microinjected with only the minimum amount of labeled protein needed to produce detectable fluorescence using the image intensifier. The time of incorporation of labeled actin into the cell's endogenous pool of actin is relatively quick and occurs in <30 min. This result is in agreement with others who have noted incorporation of microinjected actin in myocytes as short as 5 min postinjection (8) and in fibroblasts within 30 min (7). The incorporation of labeled actin may result from the exchange of actin subunits in the cell by a variety of mechanisms (19, 38). It should be emphasized that the injection of fluorescently labeled actin, especially in small injection volumes, allows for its use as a dynamic probe of both the soluble actin pool in a living cell and stabilized actin structures and occurs in <30 min. This result is in agreement with others who have noted incorporation of microinjected actin in myocytes as short as 5 min postinjection (8) and in fibroblasts within 30 min (7). The incorporation of labeled actin may result from the exchange of actin subunits in the cell by a variety of mechanisms (19, 38). It should be emphasized that the injection of fluorescently labeled actin, especially in small injection volumes, allows for its use as a dynamic probe of both the soluble actin pool in a living cell and stabilized actin structures.
FIGURE 8 Nomarski (a) and fluorescence images (b and c) of fixed cells stained with rhodamine-labeled phalloidin after 30 min of spreading. A cortical band of fluorescence is seen in a high plane of focus (b). In a low plane (c), punctate structures are visible. Rarely can linear structures (c, arrow) be seen after only 30 min of spreading. Bar, 10 μm. × 1350.

Interpretation of Actin Distribution during Spreading by FAC and Immunofluorescence

Rhodamine-labeled phalloidin and antiactin indirect immunofluorescence of whole fixed, extracted cells also reveals fluorescent punctate and linear structures associated with the substrate-attached plasma membrane. Linear structures seem to be accentuated by staining with rhodamine-labeled phalloidin as compared to antiactin. This may be due in part to the binding of phalloidin to F-actin structures in contrast to antiactin antibodies, which may bind both filamentous and residual nonfilamentous actin. It should be emphasized, however, that linear structures were seen with both techniques and that no dramatic difference between phalloidin labeling and antiactin labeling was detected. Future studies will evaluate other fixation procedures since it has been demonstrated (22, 23) that different fixation procedures can optimize the preservation of cytoskeletal elements, particularly actin filaments in thin, peripheral regions of attached cells.

The appearance of nonextractable fluorescent structures as a function of time is correlated with the degree of spreading in thereby affecting the local accessibility of a small fluorescently labeled molecule (35). Other soluble control proteins will be tested in the future to determine those which remain excluded from the nucleus in macrophages, and co-injections of both labeled controls and actin into the same cell will be performed.

The technique of FAC requires that images of the same cell be generated over a period of time without damaging the cells. The use of image intensification (21) allows for the recording of fluorescence distributions with a minimum of photobleaching damage to the cell. Therefore, although there is some sacrifice in image quality when photographing an image on the TV monitor, there is a gain in sensitivity and time resolution. Sequential images of living, functional cells would not be feasible by photographing microinjected cells directly on the microscope, even though this technique produces images of distinct fluorescent structures (7, 8, 12). Future experiments will employ image analysis methods on digitized images, thereby optimizing the quality of images produced.

FIGURE 9 Fluorescence images of fixed cells after 3 h of spreading. (a) Stained with rhodamine-labeled phalloidin. (b) Antiactin indirect immunofluorescence. Both fluorescent punctate and linear structures are visible above the attached membrane. Note that images produced by both techniques are nearly indistinguishable. Bar, 10 μm. × 1700.
FIGURE 10 (a and b) Macrophage adherent membranes stained with rhodamine-labeled phalloidin. Varying numbers of punctate and linear structures are visible. Bar, 10 µm. x 1,200. (c and d) after staining with rhodamine-labeled phalloidin, the same adherent membrane is negatively stained and examined with electron microscope. Fluorescent structures coincide with electron dense areas at the ultrastructural level (c and d, arrows). Bar, 10 µm. x 1,800.

FIGURE 11 Macrophage adherent membrane stained with rhodamine-labeled phalloidin and negatively stained and examined with electron microscope. Note that a represents only a portion of the cell which remains after shearing. The boxed area of a corresponds to the negatively stained image shown in b. The four fluorescent punctate structures represent the four electron dense foci shown in b. The foci are closely associated with meshworks of thin actin filaments (arrow). (a) Bar, 10 µm. x 1,600. (b) Bar, 0.5 µm. x 33,800.
fixed cell preparations. Cells are plated and allowed to spread for various times and fluorescent structures are quantified by scoring cells in a number of fields. Punctate structures are observed as early as 15 min after plating of cells, suggesting that actin foci are involved even for initial attachment of the cell to the substrate. In addition, a cortical band of fluorescence observed in a high plane of focus suggests that in the observed as early as 15 min after plating of cells, suggesting that in the later stages when the cells are fully spread, most of the actin staining is of punctate and linear structures associated with the attached membrane. Fluorescent linear structures (thick bars) are formed as spreading continues. The actual extension of the cell's boundary may involve the elongation of membrane-associated actin especially from foci on the cytoplasmic surface of the attached membrane.

**Figure 12** Schematic drawing summarizing our results on the organization of actin during the spreading process. During initial attachment to the substrate (A), punctate structures (bold dots) are formed. The nucleus (N) is surrounded by a spherical distribution of actin structures (thin lines) which appear as a cortical band of fluorescence in a high plane of focus. As spreading is initiated, the cell begins to flatten, thereby increasing the surface-to-volume ratio. As the cell continues to flatten (B), an increasing percentage of cytoplasmic actin comes into contact with the spreading membrane. Thus, actin which was previously associated with either unattached membrane or cytoplasm in the perinuclear region becomes associated with the substrate attached membrane. Fluorescent linear structures (thick bars) are formed as spreading continues. The actual extension of the cell's boundary may involve the elongation of membrane-associated actin especially from foci on the cytoplasmic surface of the attached membrane.

In summary, the optimal approach for elucidating the distribution of cytoskeletal and contractile proteins involved in motile processes is a combination of three techniques. Immunofluorescence and electron microscopy can yield a great deal of information concerning the structural components of the cytoskeleton while FAC allows us to follow dynamic changes of both the soluble and structural pools of cytoskeletal proteins in living, functional cells (35). Results from one technique must be interpreted with caution due to the potential artifacts and limitations of each technique. The concomitant use of FAC and immunofluorescence can minimize artifacts such as local differences in pathlengths and accessible volume, thereby permitting qualitative determinations of the local concentrations of proteins in different regions of the cell.

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