

Changing Patterns of Actin Localization During Cell Division

(fluorescent heavy meromyosin/cytokinesis/ameboid movement)

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ABSTRACT Changing patterns of actin localization have been studied on a light microscopic level by means of fluorescently labeled heavy meromyosin. The cellular distribution of actin is characterized by four major patterns, each of which corresponds to a particular phase of cell division. Long actin fibers are a prominent feature of the interphase cell. They disappear as the cell rounds up for mitosis and are replaced by a diffuse distribution of actin throughout the cytoplasm. During cytokinesis, the actin is localized predominantly in the cleavage furrow. The final shift of actin occurs after the completion of cytokinesis. At this time the actin becomes concentrated in the distal poles of the cell where pseudopods form to pull the daughter cells apart. When the daughter cells have separated, they flatten on the culture dish and the fibrous pattern of actin characteristic of interphase cells returns. All of these changes take place during the 1-hr period required for cell division.

Actin filaments are being observed more and more frequently in electron micrographs of a wide variety of nonmuscle cells (1-4). Actin, myosin, and other contractile proteins, moreover, have been isolated from many different kinds of nonmuscle cells, and it has been suggested that these proteins are responsible for various types of cell motility (4-6). In order to determine if actin is, in fact, one of the contractile proteins responsible for cell motility, it is necessary to know its location with respect to the motile regions in a cell. Ultrastructural techniques (7) allow only very small areas of a cell to be examined at one time and reconstruction of the total distribution of actin filaments in one cell is an arduous task. An ultrastructural study to detect possible changes in actin distribution during different types of motility would be an even more difficult undertaking.

The division of a cell in culture involves two types of movements: first, the actual pinching of the cell in two and second, the movement of the two daughter cells apart from one an-

other (8). The present paper describes the use of a light microscopic method for studying the localization of actin in dividing cells *in situ*. Fluorescently labeled heavy meromyosin is used as a tag for cellular actin, resulting in areas of fluorescence wherever actin is present. Groups of cells can be quickly examined to determine the overall distribution of actin. Different staining patterns are observed during various stages of cell division. The results reported here suggest that cellular actin is associated with the motile regions of the cell and is recycled to perform various functions within the cell.

MATERIALS AND METHODS

Rabbit heavy meromyosin and fibrous actin were prepared by methods described by Szent-Gyorgyi and Pryor (9). Heavy meromyosin was complexed with fibrous actin (10) and dialyzed overnight against a pH 8.5 carbonate solution (11). Fluorescein isothiocyanate (1 mg of dye per 20 mg of protein) was added to the solution, and the coupling reaction was terminated after 3 hr at 4°. The fluorescent labeled fibrous-actin-heavy meromyosin complex was pelleted at $100,000 \times g$ for 3 hr, and the fluorescently labeled heavy meromyosin was dissociated from the fibrous actin with a standard salt solution (12) containing 10 mM ATP. The fluorescent heavy meromyosin was decanted after the fibrous actin had been pelleted at $100,000 \times g$ for 3 hr. Unbound fluorescein isothiocyanate was removed from the fluorescent heavy meromyosin on a Sephadex G-25 column equilibrated with standard salt. The labeled heavy meromyosin was stored at a concentration of 1 mg/ml in a solution of 25 parts of glycerol, 74 parts of standard salt, and 1 part of antibiotics (5000 units of penicillin and 5000 μg of streptomycin per ml).

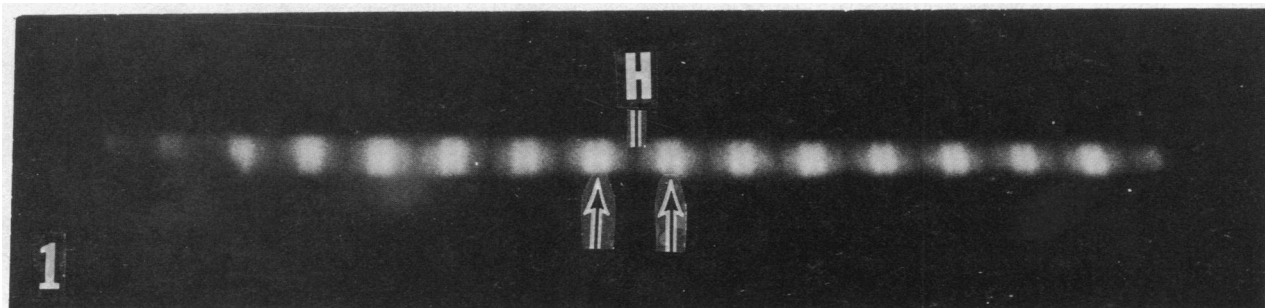
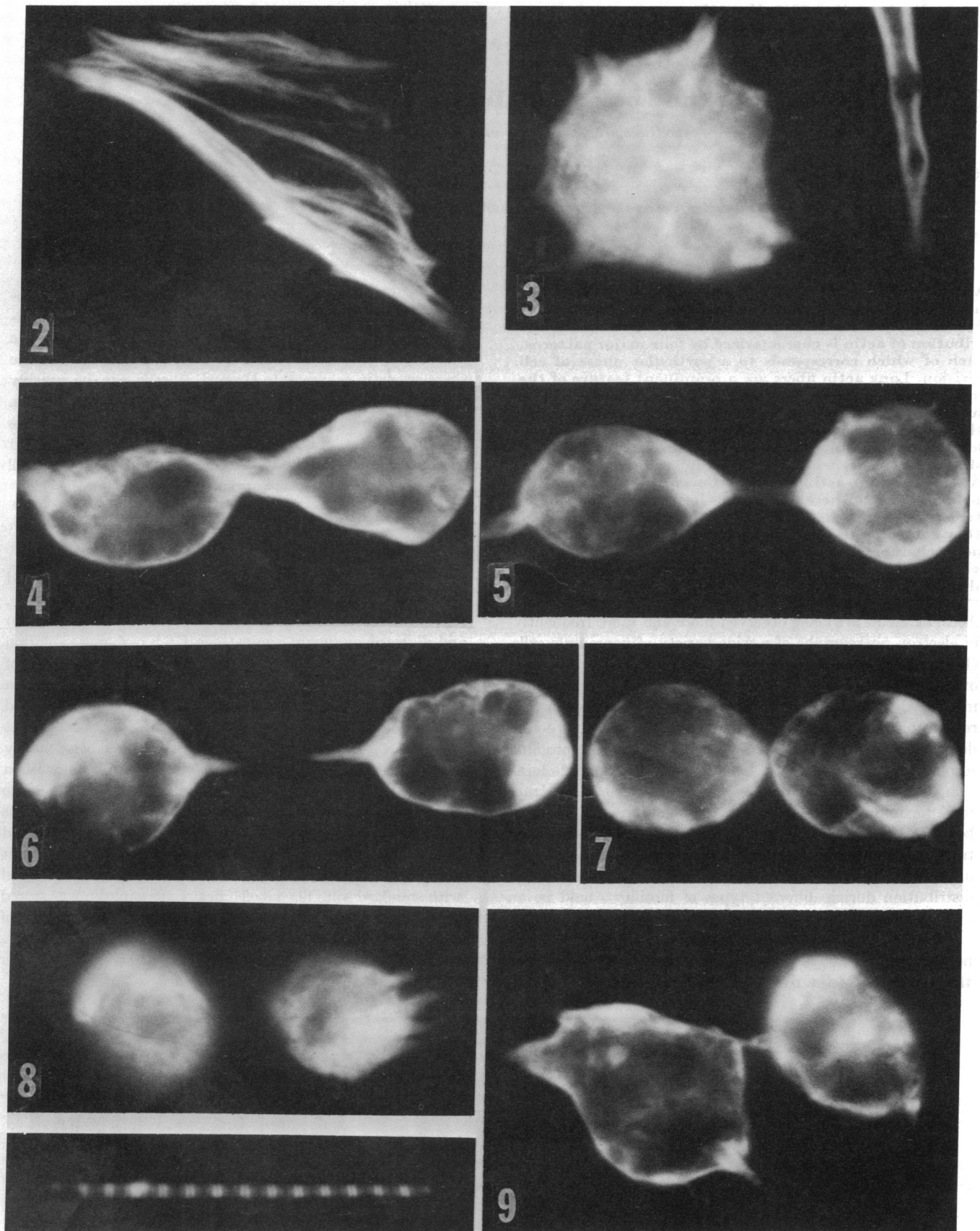


FIG. 1. An isolated myofibril stained with fluorescent heavy meromyosin. The I-bands appear as bright doublets intersected by the Z-bands (arrows), which appear as dark lines. The A-band shows some staining adjacent to the I-band in the region of thin and thick filament overlap. There is no stain in the center of the A-band (H). Magnification $\times 4000$.



FIGS. 2-9. A series of glycerinated chick fibroblasts stained with fluorescent heavy meromyosin. The myofibril at the bottom of the plate is at the same magnification as the other pictures of the dividing cells to give an idea of the relative amount of actin in the cells as well as the magnification ($\times 2300$). *Fig. 2.* Interphase cell with continuous fibers. *Fig. 3.* A cell in the process of rounding up prior to divi-

Gizzard cells were isolated from 10- and 11-day-old chick embryos and grown under conditions used for amnion cells (13). The cells were plated at low densities (50,000 cells per ml) and allowed to grow to confluency. Fibroblasts were isolated by continuous subculturing of these primary cultures. After three subculturing, the fibroblasts were plated on rat tail collagen-coated glass coverslips and placed in small tissue culture dishes. After 1 or 2 days the medium was removed and the cells were glycerinated for 1 or more days in a cold (4°), 25–50% glycerol/standard salt solution containing antibiotics (see above). The cells were then rinsed twice with a fresh glycerol solution and incubated with fluorescent heavy meromyosin overnight at 4°. Unbound fluorescent protein was removed by washing the cells with a cold standard salt solution (12). The cells were rinsed several times over a period of several hours and then stored at 4° in the 50% glycerol/standard salt/antibiotic solution. To check the specificity of the binding of fluorescent heavy meromyosin to actin, rabbit myofibrils were also stained.

RESULTS

Myofibrils

The binding of fluorescently tagged heavy meromyosin was limited to the length of the thin filaments in rabbit myofibrils (Fig. 1). Staining was most intense in the I-band. A decreased amount of staining was present in the overlap region of the A-band, but there was no staining of the Z-band or H-zone. The binding of fluorescent heavy meromyosin to myofibrils was blocked if the myofibrils were preincubated with nonlabeled heavy meromyosin or if the fluorescent heavy meromyosin was added in the presence of ATP. Myofibrils that had been labeled with fluorescent heavy meromyosin and then rinsed several times with standard salt and 10 mM ATP exhibited almost no fluorescence.

Fibroblasts

Cultures of chick fibroblasts were monitored in a phase microscope in order to record the length of time required for the completion of cell division. Cells entering division gradually lost their attachments to the surface and rounded up. In about 20 min, the rounded cells began to furrow, and after 15 min, the furrowing was complete and a mid-body could be observed. Within 5 min after the completion of furrowing, pseudopodial activity began at the ends of the cells opposite the mid-body. The daughter cells separated from one another by means of the pseudopods and then flattened on the dish. Approximately 1 hr elapsed between rounding up and the formation of two new flattened cells.

In the fluorescence microscope, fibers were observed in interphase cells running parallel to the long axis of the cell (Fig. 2). When the cells rounded up prior to division, they were attached to the dish by small spikes. These spikes were fluorescent, and there was a uniformly diffuse fluorescence over the rest of the cell (Fig. 3). No discrete fluorescent fibers could be observed in the cytoplasm of rounded cells. The next mitotic stage that could be readily identified in the fluorescence microscope was telophase. In this stage there was strong

fluorescent staining in the furrow region (Fig. 4). There was also some weak fluorescence in the rest of the cell. In late telophase, the mid-body that formed was slightly fluorescent, but most of the staining was concentrated in the furrow region (Fig. 5). After the completion of cytokinesis, there was a decreased staining of the former furrow region and a concomitant increase in staining at the opposite ends of the cells (Fig. 6). It is at these distal poles of the daughter cells that pseudopod formation occurs, enabling the cells to move apart. The pseudopods that formed in this region were also fluorescent (Figs. 7 and 8). When the separated cells began to flatten on the culture dish, staining was concentrated along the perimeter of the cells (Fig. 9). Fully flattened cells exhibited the continuous fluorescent fibers that are typical of interphase cells. Interphase cells that had been moving possessed not only continuous fibers, but also fluorescent staining in the pseudopodial region.

If the stained fibroblasts were pretreated with unlabeled heavy meromyosin or washed with standard salt and 10 mM ATP, very little fluorescence was observed in the cells. Unstained glycerinated cells were also examined in the fluorescence microscope and very little autofluorescence was observed.

DISCUSSION

The specificity of binding of fluorescent heavy meromyosin to actin has been previously documented in a variety of cell types (14). By protecting the actin-binding site of heavy meromyosin during the coupling reaction, we have overcome previous problems of nonspecific binding (15). The staining in myofibrils was limited to the length of the thin filaments. Decreased staining of the thin filaments in the overlap region of the A-band results because many of the myosin-binding sites within the A-band are already coupled with myofibrillar myosin. However, even though there is a great deal of myofibrillar myosin complexed with actin in the overlap region, fluorescent heavy meromyosin is nonetheless able to penetrate and combine with the remaining binding sites. The intensity of fluorescence is thus related to the number of myosin-binding sites that are available to the fluorescent heavy meromyosin.

The staining of dividing cells demonstrates that the cellular distribution of actin changes during the course of division. There are at least four different patterns of actin distribution during cell division (Fig. 10): (I) interphase pattern, (II) rounding up pattern, (III) furrow pattern, and (IV) cell separation pattern. Interphase is characterized by fluorescent strands that run the length of the cell, indicating that the cellular actin is localized in discrete bundles. As the cell progresses from interphase to division, the long bundles of actin fibers disappear and a diffuse staining pattern forms. The narrow spikes anchoring the cell to the substratum are also fluorescent, demonstrating that actin is present along the full length of these attachment strands.

The next major pattern of actin staining occurs when the cleavage furrow is formed. While there is some diffuse staining over the two halves of the cell, the cellular actin is concen-

sion. The cell process in the upper right is part of an interphase cell. *Fig. 4.* A cell in cleavage. *Fig. 5.* A cell that has completed cleavage. *Fig. 6.* A cell stained within 3–5 min after the termination of cleavage. Note the decreased amount of staining in the furrow region and the marked amount of staining now in the nonfurrow polar regions. *Fig. 7.* Cells that have completed cleavage. *Fig. 8.* Daughter cells that have separated and are beginning to flatten. *Fig. 9.* Flattened daughter cells.

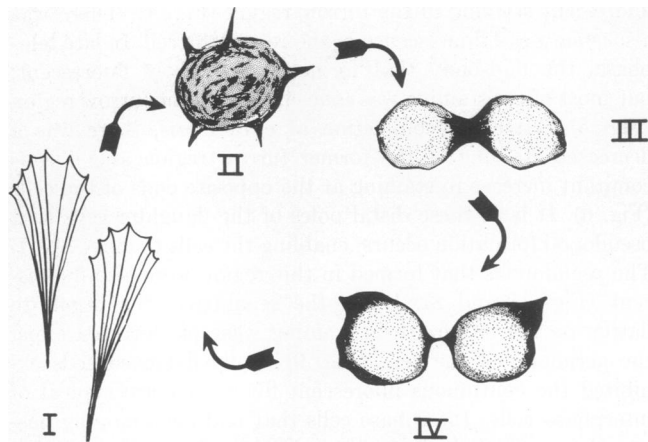


FIG. 10. A diagram summarizing the four major patterns of actin localization during cell division. Long actin fibers are a prominent feature of the interphase cell (I). These fibers disappear as the cell rounds up for mitosis and are replaced by a diffuse distribution of actin (II). During cleavage, the actin is predominantly in the cleavage furrow (III). After the completion of cleavage, the actin becomes concentrated in the distal poles, where pseudopods form to pull the daughter cells apart (IV). After separation, the daughter cells flatten and actin is again found in bundles (I).

trated mainly in the cleavage furrow. Previous ultrastructural studies (1, 2) have indicated that actin filaments are present in the cleavage furrow. However, actin filaments have also been observed in nonfurrow regions in dividing cells, and their quantitative relationship to the furrow elements has been uncertain (16). Our work has provided a picture of the total distribution of actin in cleaving cells, and it is clear that most of the cellular actin at this stage is in the cleavage furrow. Furthermore, the data presented here strongly support the equatorial constriction theory of cytokinesis postulated by a number of workers, most notably Marsland (17). These investigators proposed that furrowing is the result of contraction at the equator of the cell. Assuming that actin is necessary for this contraction, this theory would predict a concentration of actin in the cleavage furrow. Two other theories have also been proposed to explain furrowing: polar expansion and polar relaxation (reviewed in ref. 18). In polar expansion, the force responsible for cleavage is developed in the poles; the equatorial region remains passive. This theory would be supported if actin were concentrated at the poles during cleavage. This we did not observe. Polar relaxation mechanisms postulate a uniform cortical tension throughout the cell followed by a decrease in polar tension leading to cleavage (18). This theory could accommodate either a uniform distribution of actin over the cell during cleavage or an initially uniform distribution of actin followed, at cleavage, by a withdrawal of actin from the poles. Our fluorescent heavy mero-myosin staining would not support the first of these possibilities but would support the second.

After the cells have completed cytokinesis and begin to move apart from one another, most of the fluorescent staining is found at the poles where pseudopods are forming. There was very little staining in these polar regions during the cleavage furrow stage. Before the pseudopods are actually formed it is possible to identify precisely the regions of future pseudopodial activity in these immobile dividing cells. The actin becomes

localized in these regions only a very short time before movement occurs (within 5 min). This localization of actin is compatible with the view that actin is necessary for pseudopodial movement.

These results all support the growing body of evidence that implicates actin in nonmuscle contractile events. Since myosin and other contractile proteins have also been found in nonmuscle cells (4), it is very likely that actin-myosin interaction produces the contractile force in nonmuscle cells as it does in muscle cells. Just as initial methods of fixation were inadequate to preserve thick myosin filaments in smooth muscle (19), so too, present fixation methods do not permit the detection of the much smaller numbers of myosin units that may be present in nonmuscle cells (4).

The synthetic activity of dividing cells is very low (8) and, thus, it is unlikely that the different actin patterns are due to localized synthesis and breakdown. The different patterns of actin localization during cell division may be due to a recycling of actin within the cell, as illustrated in Fig. 10. As a cell progresses through division, actin may be transformed first from fibrous interphase bundles to a more uniform cortical distribution as the cell rounds up. When contraction is required for cleavage, the actin could be reorganized into a contractile apparatus which would supply the force for cleavage. This labile apparatus could then be dismantled and shuttled to the distal poles to provide contractile units for pseudopodial activity. With the return of a sessile interphase state, the actin could once more be stored in long bundles. Discovering the conditions that are responsible for the shuttling of the actin (and presumably other contractile proteins) from one site to another will be the most challenging aspect of nonmuscle motility.

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