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Antibody to Myosin: The Specific Visualization of Myosin-Containing Filaments in Nonmuscle Cells

(actin/immunofluorescence/microfilaments/cell movement)

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ABSTRACT **Myosin in human, rat, mouse, and chicken fibroblasts was localized by indirect immunofluorescence microscopy using antibodies prepared in rabbits against highly purified chicken gizzard myosin. Filaments containing myosin span the interior of the cells and are often parallel to each other. The majority of the fibers are concentrated toward the adhesive side of the cell. Most of the myosin-containing filaments show "interruptions" or "striations." From a comparison of these fibers in fluorescence and phase microscopy and from previous results on actin-containing fibers, we conclude that at least some of the cytoplasmic myosin can be found in the actin-containing fibers, which themselves have been shown to be very similar or identical to the microfilament bundles. The occurrence of both myosin and actin in the microfilament bundles provides a basis for the motility and contractility of the cell.**

Actin is assumed to be a major protein of most if not all eukaryotic cells (for a review see ref. 1). Microfilaments, one of the three major fibrous structures of a variety of eukaryotic cells (2), are thought to contain F-actin, since they can be selectively decorated with heavy meromyosin, the specific proteolytic fragment of skeletal muscle myosin known to interact with actin (3). In agreement with this assumption, Lazarides and Weber (4) have recently shown by indirect immunofluorescence microscopy that actin-specific antibodies stain well-characterized fibers in a variety of fibroblasts of chicken, mouse, rat, hamster, monkey, and human origin. These fibers have been further characterized by a combination of light and fluorescence microscopic studies and have been correlated with the microfilament system (5), in agreement with previous studies (6). Microfilaments are thought to be part of the contractile system of eukaryotic cells. However, it is generally assumed that at least in muscle neither actin nor myosin alone can generate force of movement. Therefore, if the actin-containing fibers of the microfilament are involved in cell movement, then myosin-like molecules must coexist with actin somewhere in the cytoplasm. Indeed, myosin has been found in various cells already known to contain actin (for a review see refs. 1 and 7). So far no unambiguous localization of myosin in fibroblastic cells has been achieved, and it is not known which form of organization myosin molecules have in a living fibroblastic cell.

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We have used antibody against highly purified gizzard myosin in indirect immunofluorescence microscopy on fibroblasts of human, mouse, rat, and chick origin. In all cell lines studied so far, the antibody decorates a complex network of fibers, which, in contrast to the actin fibers described by Lazarides and Weber (4), show "interruptions" or "striations." Since a majority of fibers can be observed by phase and fluorescence microscopy, we propose that microfilaments contain both actin and myosin.

MATERIALS AND METHODS

Cells were grown on glass coverslips in the appropriate medium. The medium was removed and the coverslips were incubated for 15–20 min at room temperature in 3.5% formaldehyde in phosphate-buffered saline. The coverslips were subsequently washed in the buffered saline and treated with absolute acetone at −10° for 5 min. After air drying the coverslips were treated essentially as previously described (4). The rabbit antibodies to gizzard myosin were previously purified by ammonium sulfate precipitation and chromatography (4) and were stored in 0.02 M Tris·HCl, pH 7.8, 0.15 M NaCl at −20° at a concentration of 10 mg/ml. For fluorescence microscopy a 1:15 dilution of the γ-globulins in phosphate-buffered saline was used. The fluorescein-labeled goat antibodies to rabbit γ-globulins were obtained from Miles Co. and used at a 1:10 dilution in the buffered saline. Coverslips were viewed through oil in a Zeiss PM II microscope with ultraviolet optics using epifluorescence at 63- or 100-fold magnification.

The following cell lines were used. 3T3, an established mouse line (8), was grown in Dulbecco's modified Eagle's medium with 10% calf serum. The human skin fibroblasts, Bo Mat, were obtained from the American Type Culture Collection and were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum.

RESULTS

Myosin was purified from chicken gizzards by a procedure to be published in detail (U. Groeschel-Stewart and K. Weber, in preparation). Briefly, actomyosin from gizzard was dissociated with $Na_4P_2O_5$ in the presence of magnesium and ATP followed by ammonium sulfate precipitation. Since the myosin fraction was still contaminated by traces of actin, the preparation was further purified by chromatography on Sepharose in the presence of 0.6 M KCl, 1 mM ATP, 5 mM dithiothreitol in 0.02 M Tris·HCl, pH 7.5. The myosin prep-

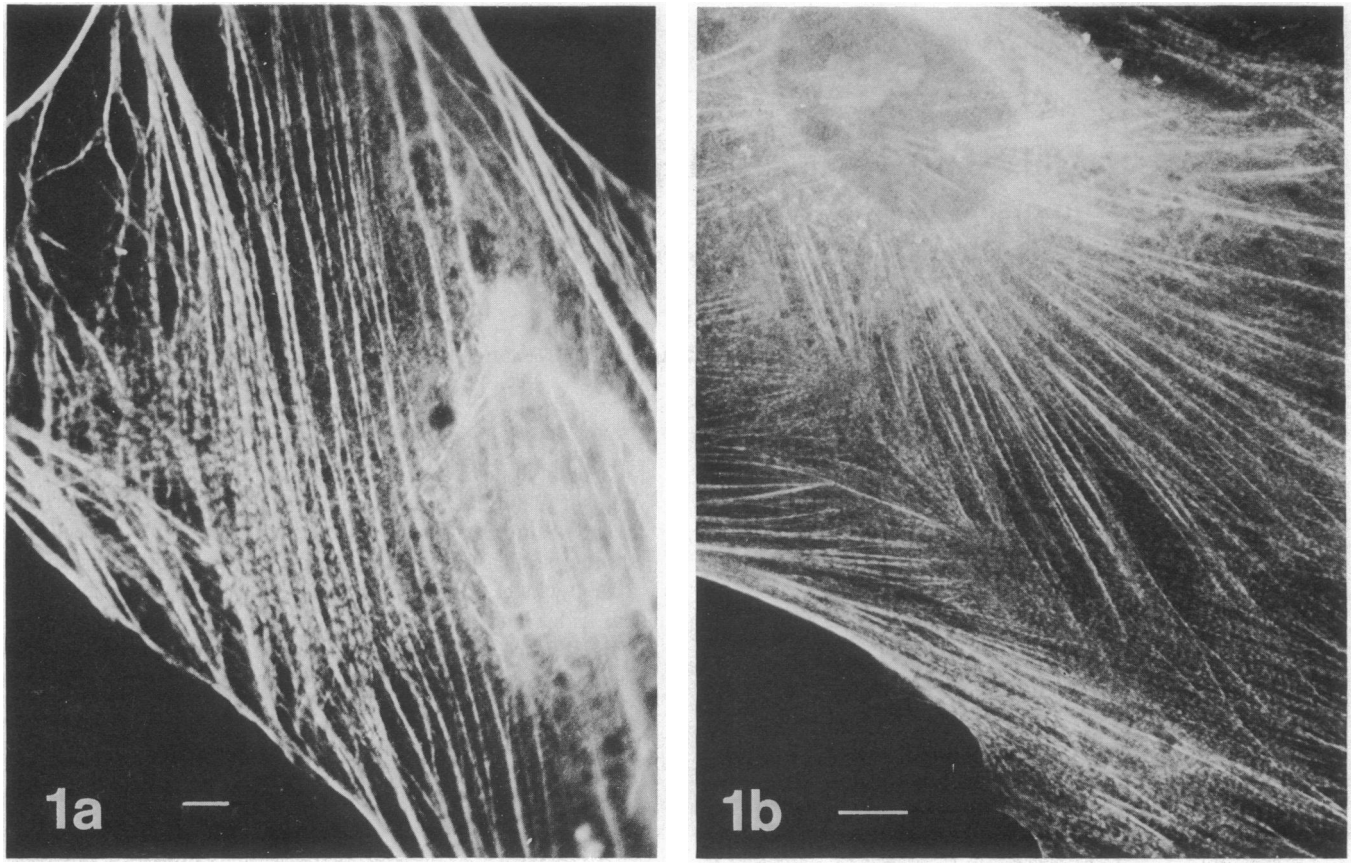


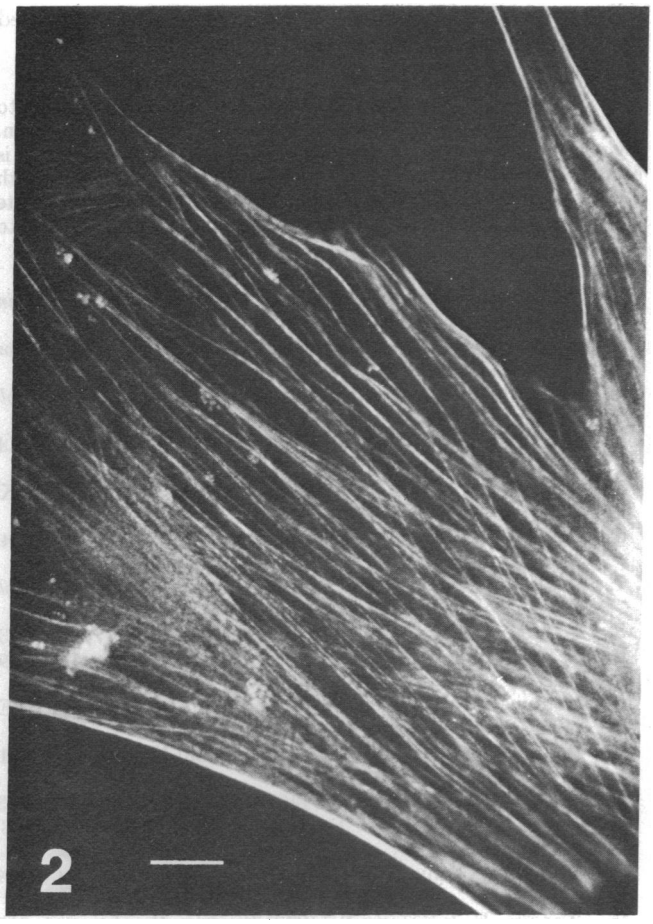
FIG. 1. Indirect immunofluorescence with antibody to gizzard myosin. (a) Part of a mouse 3T3 cell; (b) part of a human fibroblast cell (Bo Mat). The length of the bar indicates 10 μm.

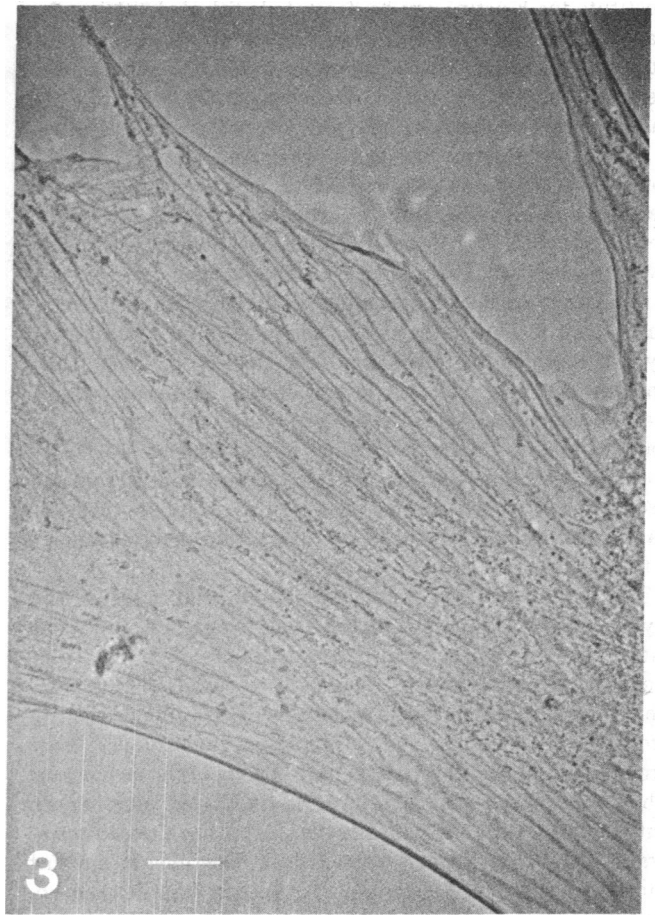
aration obtained was freed of nucleic-acid-containing material by batchwise treatment with Whatman DE 52 DEAE-cellulose followed by precipitation with dilute buffer at pH 6.0. The gizzard myosin obtained was subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and showed the typical myosin heavy chain and two light chains of different molecular weight (for a review see ref. 1). Actin and tropomyosin could not be detected on the same gels. The myosin preparation was used to obtain antibodies in rabbits by the procedure of Groeschel-Stewart (9). The antibody reacted in immunodiffusion with gizzard myosin and a fraction of human platelets enriched in myosin as well as with human uterus actomyosin. The antibody failed to react with actin from chicken muscle or with myosin from human or chicken pectoral muscle. Details of these studies will be published elsewhere (U. Groeschel-Stewart, in preparation).

Cells grown on coverslips were fixed in formaldehyde for optimal preservation of fibrous material and then stained as described in *Materials and Methods*. Fig. 1 show the fluorescent pattern with two different fibroblastic cells: the mouse 3T3 line and the human skin fibroblast Bo Mat. The fluorescent staining reveals a multitude of myosin-containing filaments. They span the interior of the cell and frequently run parallel to one another for a long intracellular distance parallel to the long axis of the cell. Occasionally the cell periphery is clearly outlined. The majority of these fibers appear to lie toward the adhesive side of the cell, since they are in focus only when the edge of the cell is also in focus. Different cells on the same coverslip have a high degree of individuality although the general features of the myosin structure described above are always recognizable.

The pattern of fibers visualized is extremely similar to that previously reported for actin staining using actin-specific antibody (4). There is, however, one remarkable difference, which becomes more obvious in epifluorescence at higher magnification. The myosin-containing fibers, unlike the actin-containing fibers (4), show "interruptions" or "striations," indicating that the fibers contain the myosin in an interrupted arrangement.

In order to determine if the myosin-containing structures can also be recognized by phase optics, we photographed a single antibody-stained 3T3 cells by fluorescence optics and then examined the same cell by phase contrast microscopy (Figs. 2 and 3). There is a convincing relation between "uninterrupted fibers" visualized in phase microscopy and "interrupted fibers" recognized in fluorescence microscopy. These results, together with previous data on actin-containing fibers (4, 5) and their relation to fibers visualized in living cells (5), indicate that the antigen stained by indirect immunofluorescence with antibodies to gizzard myosin is localized in or in close proximity to the actin-containing fibers. It should be pointed out that in Figs. 1 and 2 not all the myosin-containing fibers show the "striations" revealed in the majority of fibers visualized. This finding may be due in part to being unable to focus on all fibers simultaneously, thus giving the impression of some fibers without "striations." Furthermore, even with epifluorescence recognizable "stri-





FIGS. 2 and 3. The same 3T3 cell (part) after fixation and staining with myosin antibody viewed by fluorescence microscopy (Fig. 2) and phase contrast (Fig. 3). The length of the bar indicates 10 μm.

ations" are often difficult to record photographically and are clearly demonstrated only in very well spread cells. However, we can also not rule out that some "striated" staining occurs in regions of the cell where no noticeable phase-dense fibers occur. This may be partially due to structures that are not thick enough to be recognized in phase. Alternatively we may be sometimes observing in these areas a "ground plasma-like" structure devoid of thick fibers as described by electron microscopy studies, in protozoa cytoplasm (10) or blood platelets (11).

DISCUSSION

It has been argued previously that the major fibrous structure revealed by phase optics in well-spread 3T3 cells involves a structure similar or identical to the microfilament system revealed by electron microscopy (5). This conclusion was based on the finding that the fibers visible by phase optics as "stress fibers" in living cells show a pattern and display similar to the immunofluorescent pattern obtained in fixed cells in indirect fluorescence microscopy using actin-specific antibodies (4, 5). The results obtained in this paper argue strongly that the antigen stained with gizzard myosin antibody is also localized in fibers and that some of these fibers, at least in antibody-treated cells, can clearly be observed with phase optics (see also *Results*). Since the display of these particular fibers is very similar to those previously found using actin antibody, we propose that at least some of the myosin of the cell is localized in the microfilament system. There is, however, an obvious difference in the immunofluorescent pattern displayed in fibroblasts with actin antibody and with myosin antibody. After staining with actin antibody, fibers are visualized (4, 5) that run long distances inside the cell without any interruptions recognizable by fluorescence microscopy. Myosin antibody, on the other hand, reveals "interruptions" or "striations" clearly recognizable at high resolution in the microscope. Given the resolution of fluorescence microscopy and the inherent difficulty in interpretation of results obtained by indirect immunofluorescence in quantitative terms, we are currently not able to decide between different arrangements of myosin in relation to actin structures in the microfilament system. The myosin structures may be (*a*) interspersed between different F-actin fibers, (*b*) predominantly localized in relation to one or the other actin filament, (*c*) connected to another yet-unidentified structure within or separated but close to the microfilament, or any combination of the above. The results obtained so far invite the interpretation that actin and myosin may be sufficiently close in fibrous structures, which at least in well-spread cells are identified as microfilament bundles, so that these fibers could indeed act as a contractile element of fibroblasts.

The conservative character of the protein structure of actins and to a lesser extent of myosins of all eukaryotic cells is very clearly documented by the fact that all myosins can react with skeletal muscle actin and all microfilaments (as

substitute for F-actin) can be decorated with skeletal muscle heavy meromyosin (for a review see ref. 1). Accepting both actin and myosin as part of the microfilament system opens the question of which of the other proteins involved in contraction are organized in this structure of nonmuscle cells. In all muscle systems that have so far been studied, regulation of the interaction between actin and myosin is Ca^{++} mediated, either through the troponin–tropomyosin complex or through Ca^{++} sensitivity of the myosin itself (for review see ref. 12). The existence of tropomyosin-like proteins in some nonmuscle cells including brain, platelets, and fibroblasts (see ref. 1), together with the report of an actin–tropomyosin complex in fibroblasts (13), makes it likely that tropomyosin may also be found in microfilaments. Since there is very good evidence for the presence of troponin in platelets (14) and indirect evidence for its presence in *Physarum* (15), it is possible that the microfilaments of fibroblasts also contain a troponin-like system. If this should be the case, Ca^{++} control should be found for contractility and movement of fibroblasts.

The experiments presented here argue that most of the myosin of fibroblasts is of cytoplasmic origin, in agreement with previous biochemical fractionation experiments (7). However, the data does not exclude the possibility that myosin is also part of the plasma membrane. Using immunological procedures, Willingham *et al.* (16) and Gwynn *et al.* (17) have presented evidence that myosin may be part of the membrane. They have shown that myosin could be labeled with myosin antibody from the outside without disrupting the cell membrane (16, 17) and our unpublished experiments support this conclusion (R. Pollack and K. Weber, unpublished observations).

In conclusion, the results obtained in this paper establish that at least some of the myosin of the cell is localized close to the actin, which is organized in microfilament bundles. This result establishes that actin and myosin are close enough in the cell to provide the basis for contractility and movement exerted by the action of actin and myosin.

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