Modulation of Cellular Morphology and Locomotory Activity by Antibodies against Myosin

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Abstract. Three monoclonal antibodies directed against chicken brush border myosin were used to study the possible function of myosin in microfilament organization and locomotion of chicken fibroblasts. These antibodies bind to distinct and separate epitopes on the heavy chain of chicken nonmuscle myosin and display differential effects on myosin filament formation and actin-myosin interaction (Citi, S., and J. Kendrick-Jones. 1988. *J. Musc. Res. Cell Motil.* 9: 306–319). When injected into chicken fibroblasts, all antibodies induced breakdown of stress fibers. Concomitantly, a large proportion of the cells developed extensive lamellae which altered their morphology drastically. These cells showed also increased locomotory activity. All effects were concentration dependent and reversible. The most drastic alterations were observed with cells injected with antibody quantities exceeding the quantity of cellular myosin (molar ratios of antibody to myosin >3:1). The finding that antibodies with different effects on myosin filament formation in vitro all induce similar intracellular processes suggests that it is the antibody-induced decrease in functional myosin that triggers an increase in plasma membrane dynamics and locomotory activity, rather than differences in myosin filament length or conformation.

Among the numerous members of the family of microfilament proteins, myosin claims an especially important position: It is both a structural component and an enzyme, converting chemical into physical energy. The "classical" myosin molecule consisting of two heavy chains and four light chains (cf. reference 28) is of a characteristic asymmetry (11) and carries discrete domains which can be associated with different functions: The rodlike tails are able to form ordered aggregates (19) while the twin heads act as mechano-enzymes (28) comprising the crossbridges which interact with actin. Myosins of this type have been identified in a wide variety of species and tissues (cf. reference 32).

With the exception of striated muscle myosin, the details of the role myosin plays in various motile processes have remained elusive until now. In the case of stress fibers, it seems reasonable to assume that the observed bipolar arrays of myosin may not only be structural elements of these microfilament bundles (cf. reference 27) but may also be responsible for the tension generated by them (20, 26). If this is so, then the stress fiber-bound myosin would develop force within the plane defined by its long axis, analogous to the situation in the sarcomere. Similarly, the findings that myosin is localized in the cleavage ring (14, 37) and that cleavage can be affected with antibodies against myosin (29) suggests that this protein provides the force for cytokinesis. The role of myosin in locomoting cells that usually express no or only a few stress fibers (cf. reference 2), but frequently develop a polarized shape and large leading lamellae, is not at all clear. The observation of living, locomoting cells in culture shows that many different motile processes take place during locomotion, i.e., ruffle and microspike formation at the leading edge, rapid spreading of the front part of the cells to large leading lamellae, development and severing of retraction fibers, and shape changes including reorientation of the long axis of the cell from a horizontal to a transversal position with respect to the direction of locomotion (cf. references 1, 16, 34). If these processes also depend on actomyosin interactions, myosin must be able to exert force in various planes within three-dimensional actomyosin networks, and its interaction with actin filaments must be controlled separately in the different compartments of the cell.

A valid image of the role myosin plays in different aspects of motility may be obtained by decreasing the intracellular myosin and subsequently analyzing the consequences of this manipulation. This approach has been used successfully by several groups. To study the role of myosin in locomotion and development, the expression of myosin in the cellular slime mold *Dictyostelium discoideum* has been interfered with in two ways: by introducing into slime mold amebae a mutant gene coding only for the amino-terminal portion of the heavy chain (10), and by introducing a cloned sequence

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of the myosin heavy chain under conditions in which an antisense RNA transcript is produced, leading to the elimination of the normal transcript by intracellular hybridization (25). Both techniques reduced the amount of the normal myosin heavy chain in these cells very effectively. Another approach, aimed to study the role of myosin in cytokinesis, was based on microinjection of a polyclonal antiserum against starfish myosin into developing starfish embryos (24, 29).

In this article we report results obtained with chicken embryo fibroblasts injected with three monoclonal antibodies which bind to different epitopes on the heavy chain of cellular myosins (3, 4, 23). Our results show that microinjection of such well-defined antibodies leads to antibody-induced changes in cell shape, microfilament organization, and locomotory activity. These findings are discussed in relation to the alterations of cellular motility triggered by genetic surgery in more primitive cells as mentioned above (10, 25).

Materials and Methods

Cells

Chicken embryo fibroblasts were obtained from fertilized eggs between days 9 and 11, and grown in DME (Gibco Laboratories, Eggenstein, Federal Republic of Germany) supplemented with 7.5% FCS. For microinjection experiments, cells were trypsinized and seeded onto sterile glass coverslips carrying loops drawn with a diamond pencil. These loops of various shape allowed for unequivocal identification of injected cells later on. The cells were kept on glass for at least 36 h before using them in microinjection experiments.

Antibodies

Monoclonal antibodies were raised against chicken brush border myosin (3, 4, 23): BM1 which binds one-third, BM2 which binds two-thirds of the way from the tip of the tail, and BM4 which binds at the tip of the tail were used in the experiments reported here. For control experiments, rabbit anti-mouse IgG was affinity purified on mouse IgG bound to CNBr-Sepharose (Pharmacia, LKB GmbH, Freiburg, FRG).

All antibodies were concentrated by centrifugation (Centricon concentration tubes; Amicon Corp., Witten, FRG) or pressure dialysis (collodium bags; Sartorius Filters, GmbH, Göttingen, FRG), to concentrations between 1 and 30 mg/ml. For microinjection, all antibodies (in PBS) were sterile filtered using microfilters (Millipore GmbH, Eschborn, FRG).

Microinjection Experiments

Microinjection was carried out according to reference 15, and microcapillaries were drawn from glass capillaries (type GC 100F-10; Science Products Trading GmbH, Freiburg, FRG). Microcapillaries with tip openings of ~1 μm were filled with the help of a "filling capillary" (x-ray capillaries; Glas GmbH, Berlin), and operated with a Leitz micromanipulator under optical control using a Zeiss inverted microscope. The tip of the microcapillary was placed into the perinuclear area of the cells in culture dishes. Pressure was generated either by a hand-operated syringe or an Eppendorf Injector (Eppendorf Gerätebau, Netheler und Hinz, Hamburg, FRG). The injected volume was controlled by the time period allowed for contact between the microcapillary and the injected cell. On the average the injected volume was 10^{-7} μl (12). After injection the cells were placed back into the incubator for various time periods.

Fluorescence Microscopy

Indirect immunofluorescence was carried out with chicken embryo fibroblasts that were either permeabilized with 0.5% Triton X-100 in PBS (140 mM NaCl, 2.6 mM KCl, 8 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.2), and then fixed with 3% formaldehyde in PBS, or directly fixed with methanol at −20°C, reacted with the specific antibody, and subsequently with a second antibody (rabbit anti-mouse), coupled to sulforhodamine B (Polysciences Ltd., St. Goar, FRG) rhodamine or to FITC. Actin organization in injected cells was revealed by staining formaldehyde-fixed, Triton X-100–extracted cells with rhodamine-phalloidin (Faulstich, H., Max Planck Institute, Heidelberg). To reveal the cellular distribution of the microinjected antibodies, cells were fixed and incubated with a second, FITC-coupled antibody (sheep anti-rabbit IgG or rabbit anti-mouse IgG, both affinity purified). Cells were examined with a Zeiss Ultraphot or Axiophot microscope, equipped with epifluorescence.

Determination of Shape Changes and Locomotory Activity

To follow changes in morphology and locomotory activity of injected cells during a period of several hours, dishes were periodically observed under phase optics. Injected cells were relocated by determining their position in relation to the diamond loops, they were then photographed under low intensity light, and placed back into the incubator. By superimposing the photographs, using the diamond marks on the coverslips as coordinates, the same cell was relocated, and the distance between the position of the nucleus in the photographic sequences was determined.

Results

The monoclonal antibodies BM1, BM2, and BM4 raised against chicken brush border myosin have been described previously (3, 4, 23). All three antibodies bind to the rod portion of the myosin heavy chain. Epitope localization of these antibodies and their influence on myosin filament formation are compiled in Table I. It can be seen that in vitro, these antibodies have quite different effects. Therefore, we were interested to study their effects on myosin and on myosin-based motility in living cells. When tested in indirect immunofluorescence, they reacted strongly with chicken, but only weakly with a variety of mammalian cells. Therefore, we chose chicken embryo fibroblasts for microinjection experiments.

We injected these antibodies in quantities sufficient to compensate all intracellular myosin. For this purpose, we calculated the amount of antimyosin to be injected on the following basis. Myosin comprises 0.5–1.5% of total fibroblast protein (5) which is roughly 100 mg/ml. By injecting 10^{-7} μl, we diluted the antimyosin 1:10. To obtain an antibody/myosin ratio of at least 3:1, which had been shown to give maximum effects on myosin filament assembly, the antimyosin concentration in the injection solution should be in the range of 4.5–13.5 mg/ml.

For injection, we originally used chicken embryo fibroblasts at the 10th passage. In nonconfluent cultures, >85% of such cells displayed a morphology as expected for normal fibroblasts, and numerous microfilament bundles (stress fibers). BM1 antibody, injected at a concentration of 8 mg/ml, accumulated at stress fibers and within the perinuclear area as soon as 10 min after injection, as seen in double-label experiments with rhodamine-phalloidin and FITC-coupled second antibodies (Fig. 1, *A* and *B*). Cells that were fixed 60 min after injection showed areas free of stress fibers and a more homogeneous distribution of the antibody (Fig. 1, *C* and *D*). At 4 h after injection, many cells had lost their stress fibers completely, and the antibody had concentrated in patches at the rear end of the cell (Fig. 1, *E* and *F*). 24 h after injection, the antibody was still located there, while stress fibers reappeared as thin, short bundles predominantly in the perinuclear area (Fig. 1, *G* and *H*). Control cells, which were injected with affinity-purified rabbit anti-mouse IgG (13 mg/ml) showed no disruption of stress fibers, and the injected antibody was seen diffusely distributed throughout the cell for at least 24 h (not shown).

*Table I. Comparison of the Reactivity of the Monoclonal Antibodies BM1, BM2, BM4, with Chicken Brush Border Myosin In Vitro**

Antibody	Binding site on myosin heavy chain	Influence on conformation
BM1	Rod, 2/3 of the way down from the neck region	Unfolds 10S monomer reduces filament length to 25%
BM2	Rod, 1/3 of the way down from the neck region	Reduces filament length to 60%
BM4	Tip of tail	Inhibits filament formation and disassembles filaments to bipolar oligomers

* cf. references 3 and 4.

Concomitantly with the loss of stress fibers, many of the injected cells were seen to change their shape. At the periphery, lobe-shaped protrusions developed at 60 min after injection and increased in number and size during the next 3–7 h. This process led to rather bizarre looking cells with very irregular contours. These cells had lost the sail-shaped or polygonal morphology seen in control cultures and assumed a variety of abnormal shapes including ribbon-like outlines with ragged margins, and actin-containing microspikes and small ruffles. Examples of such cells are given in Fig. 2. A close inspection of the living cells showed that their marginal regions were highly dynamic, showing rapid waves of advancement and withdrawal. Tiny dark spots at the very periphery probably consisted of small ruffles and microspikes, and new membrane eruptions were usually close to such areas. Extensive, veil-like ruffles were absent, as was also confirmed by scanning electron microscopy (not shown).

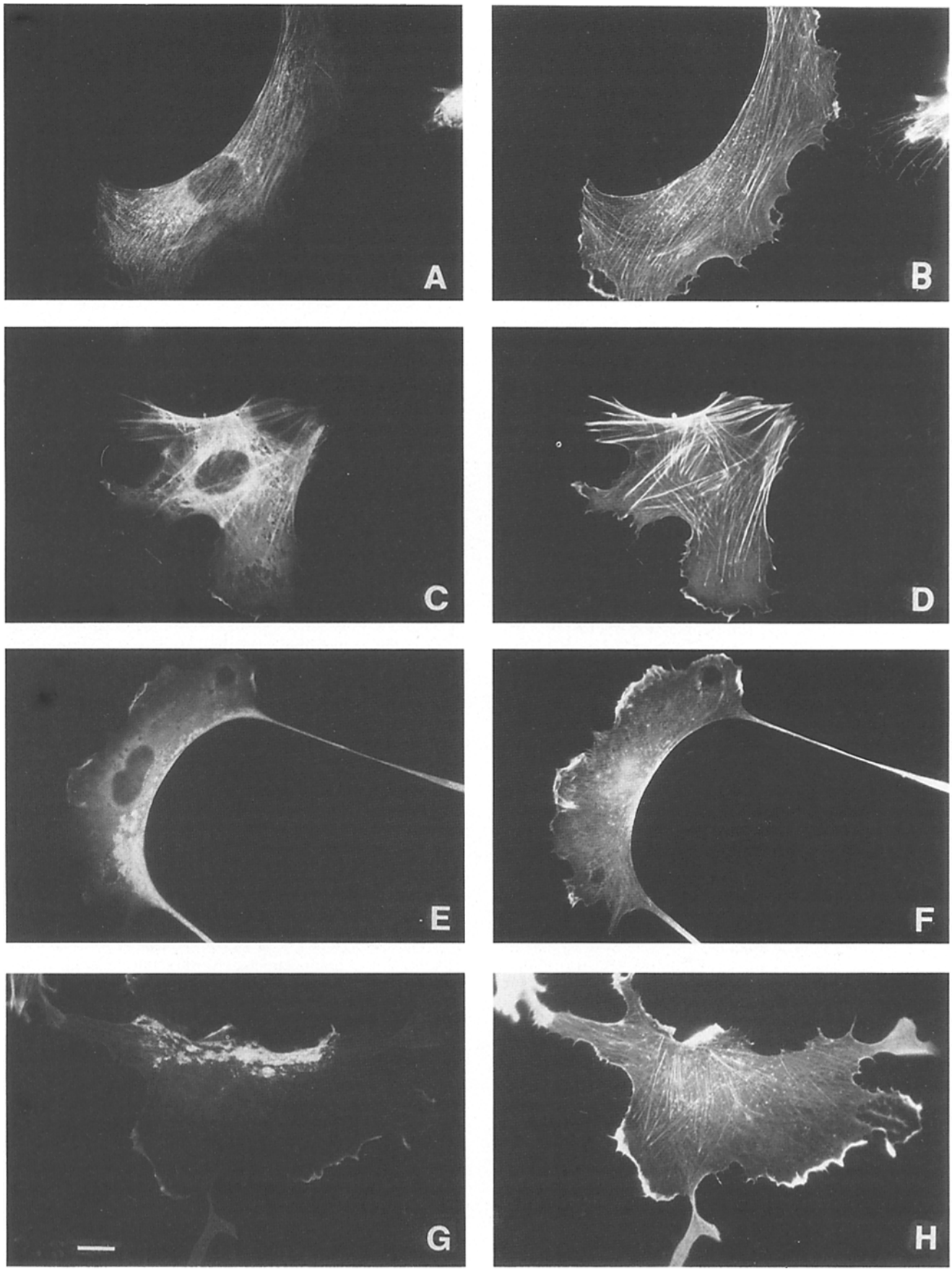
A quantitative analysis of the BM1 injected cells yielded the following results. The proportion of cells responding in the manner described above depended on their age in tissue culture. For example, the majority of cells at the 10th passage reacted this way while, on the average, only 20% of cells in their fourth passage responded to BM1 injection with these symptoms (Fig. 3). The kinetics, however, were similar for cells of all passages: the number of altered cells was highest at 8 h after injection, and then gradually decreased again (Fig. 3). The effect was also dependent on the amount of BM1 injected: when lower concentrations were used, fewer cells responded, and the recovery was faster (Fig. 4). Cells injected with 8 mg/ml needed up to 48 h before all cells had reestablished a normal fibroblastic morphology and a full stress fiber complement, as seen by phalloidin staining (not shown).

The development of multiple peripheral lamellae and the loss of stress fibers in injected cells suggested that these cells might exhibit a higher locomotory activity than control cells. This could be demonstrated by measuring travel distances of cells under phase optics. Fig. 5 shows sequences of cells injected with a control antibody (*left*) or with BM1 (*right*). Single frames were taken over a period of 11 h. While the control cell showed only moderate shape changes and almost no translocation during this period, the BM1-injected cell demonstrated the development of an abnormal cell shape and covered a much longer distance. By measuring the positions of the cell center with regard to a reference point at the diamond loop (Fig. 5, *arrows*), we calculated an average translocation speed of ~1 μm/h for the control cell and 8 μm/h for the BM1-injected cell. To obtain average values of travel distances for both types of cells, experiments were performed where the positions of cells were determined at the beginning and the end (14 h after injection) of each series. In such analyses, the actual migration tracks may be longer than reflected in the distances determined this way, since changes in direction are not accounted for. Thus, with this method, we can only detect minimal distances. Fig. 6 shows that within 14 h, BM1-injected cells, on the average, covered a minimal distance of 190 μm, and control cells ~70 μm. If one assumes that no changes in direction occurred, these values would correspond to an average traveling speed of ~14 and 5 μm/h, respectively. This result implies that the single cells observed in Fig. 5 were slower than average. However, both methods indicate that indeed there is an increase in locomotory activity as a result of BM1 injection.

As shown in Table I, BM1 had been characterized as an antibody directed against an epitope ~2/3 down the length of the rod-shaped tail of the myosin heavy chain (3), and binding of this antibody to myosin molecules has been found to unfold the 10S monomer (4, 23). It was therefore tempting to speculate that this process was the basis of the increased membrane dynamics and locomotory activity seen in cells injected with this antibody, since unfolding of myosin molecules might induce the formation of myosin oligomers (or even filaments) possibly needed for such motility processes. To test this hypothesis, we injected the antibodies BM2 and BM4. These antibodies bind to epitopes on the myosin molecule different from the one recognized by BM1 (cf. Table I). We found that with similar concentrations (8–9 mg/ml), all three monoclonal antibodies promoted the same cellular changes (Figs. 6 and 7). Thus, no difference in the effect on living cells was seen between antibodies binding to different epitopes on myosin molecules, although they had different effects on myosin filaments in vitro. We did, however, find a difference in intracellular distribution of the injected antibodies as revealed by staining with a second antibody. While BM1 caused large aggregates that accumulated exclusively in the posterior region of the cell, BM2 and BM4 were seen distributed almost homogeneously throughout the cell, including the lamellar regions (compare Fig. 1 *G* with Fig. 8 *A*).

Discussion

Our results show that the introduction of myosin-specific antibodies can change stress fiber expression, membrane dynamics, and locomotory behavior of the manipulated cells. Since the antibodies used in this study were specific for chicken nonmuscle myosin, we had to inject chicken cells. However, two lines of evidence suggest that the effects described here are not cell type or species specific. (*a*) We found reversible stress fiber disruption in rat fibroblasts injected with a polyclonal antibody reacting with the light chains of mammalian fibroblastic myosin (17, 21). (*b*) The development of abnormal shape, correlated with an increase of locomotory activity, was seen in PtK_2 cells injected with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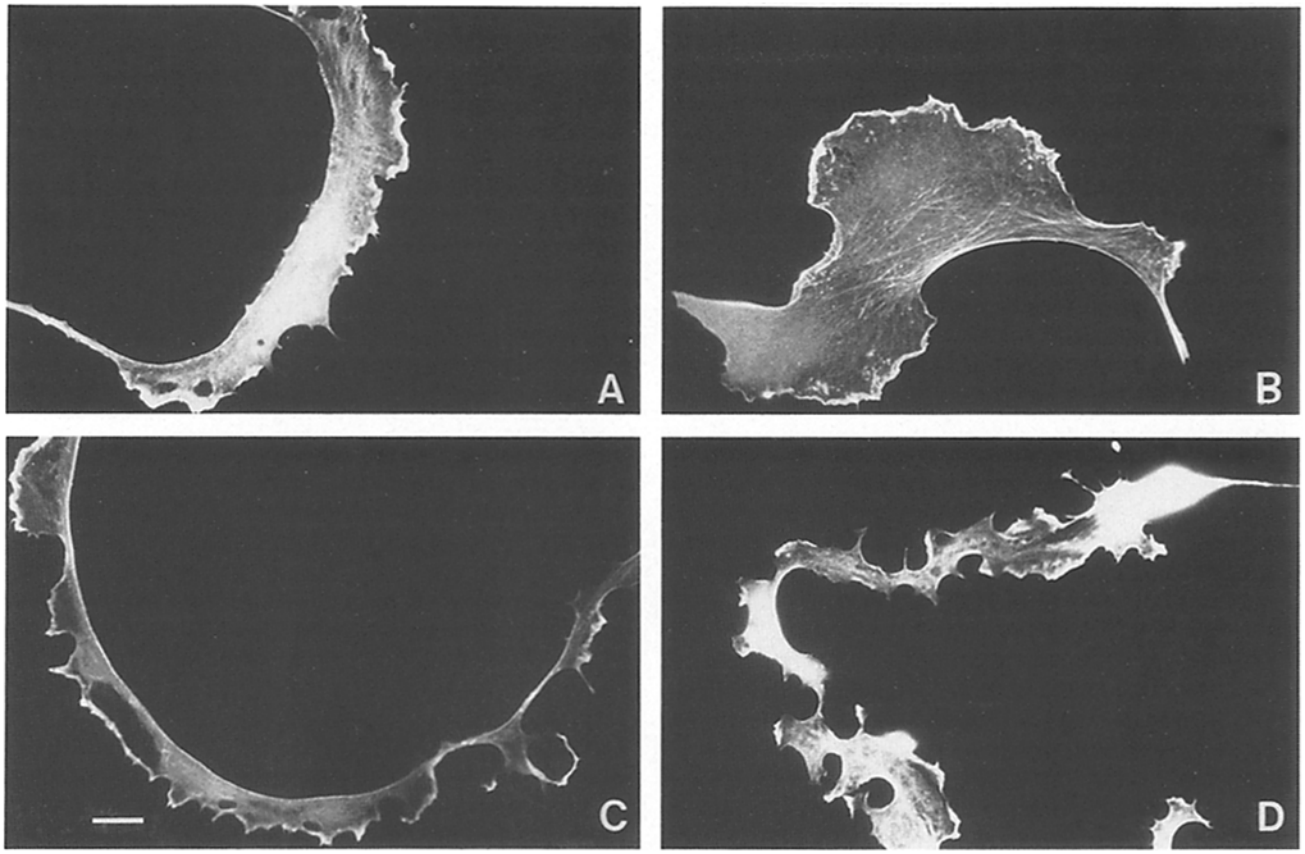


Figure 2. Four cells showing extreme shapes and bizarre looking lamellae at 8 h after injection with BM1 (8 mg/ml). Cells were stained with rhodamine-phalloidin. Bar, 10 μm.

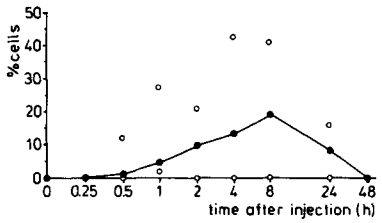


Figure 3. Increase of the number of cells without stress fibers and with altered morphology as a function of time after injection of BM1 (8 mg/ml). Each point represents the average value of 80–350 injected cells. The open circles mark the extreme values found in single experiments with at least 10 injected cells. In this series, only values obtained with cells from the fourth passage are shown.

several monoclonal antibodies directed against different epitopes of porcine brain myosin (Zurek, B., and B. M. Jockusch, unpublished data). On the other hand, the observed phenomena depended to some extent on the physiology of the

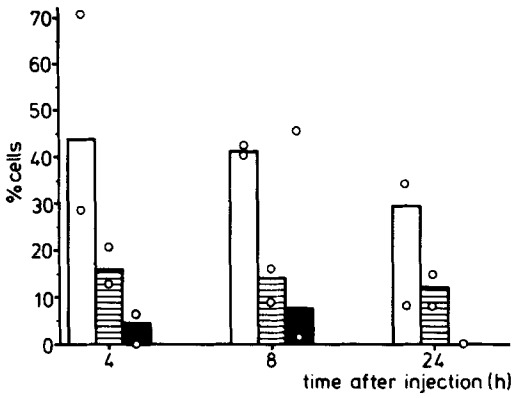
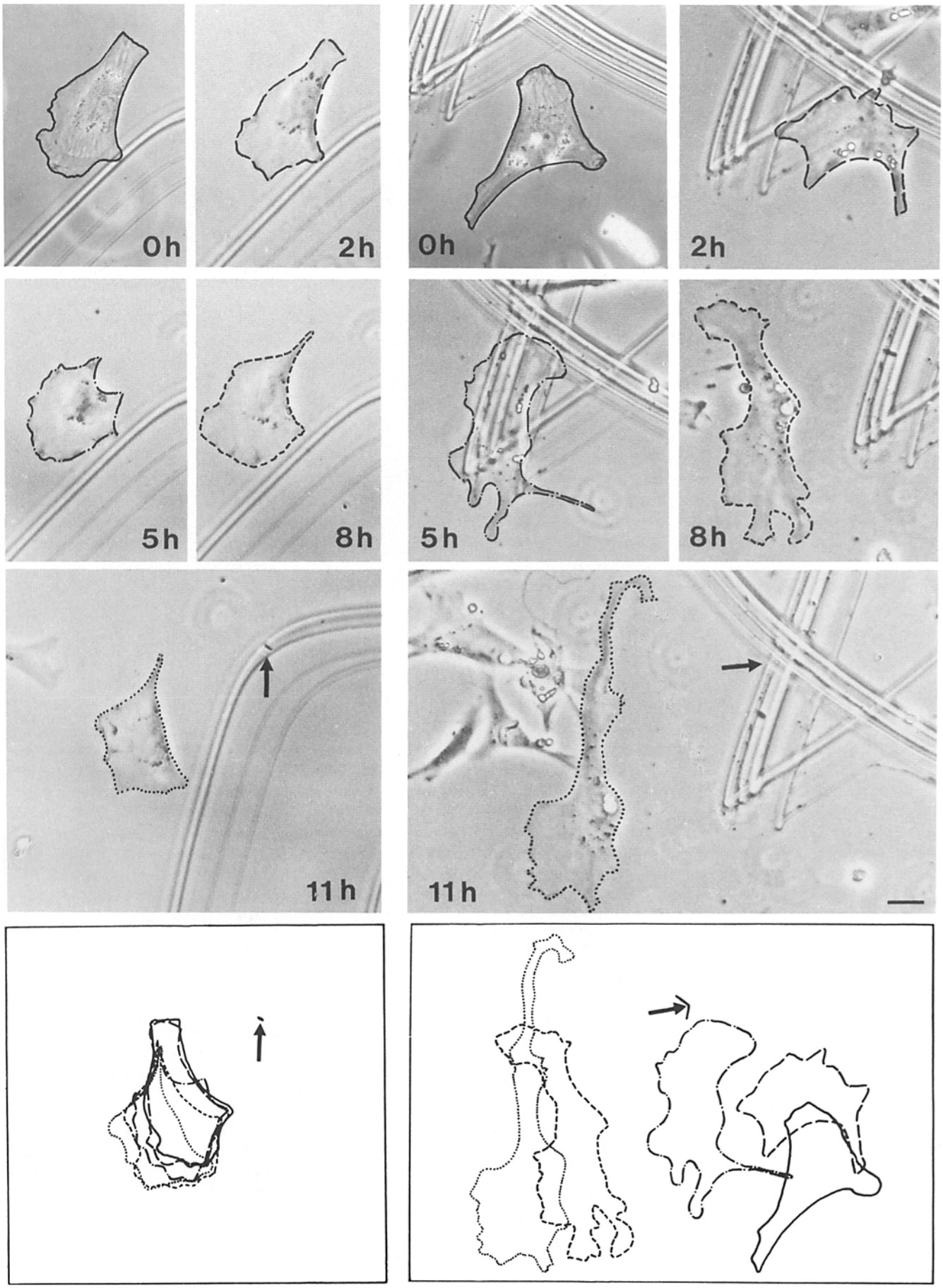


Figure 4. Increase of the number of cells without stress fibers and with altered morphology as a function of the concentration of the injected antibody (BM1). Cells at the 10th passage were injected. (*Open bars*) 8 mg/ml; (*striped bars*) 4 mg/ml; (*solid bars*) 2 mg/ml. The bars represent the summarized average values of all experiments. (*Open circles*) Extreme values found in single experiment with at least 10 injected cells.

Figure 1. Stress fiber disruption and the distribution of antimyosin in chicken fibroblasts injected with BM1(8 mg/ml), as seen in double fluorescence experiments. (*A*, *C*, *E*, and *G*) Distribution of the injected antibody as revealed by FITC rabbit anti-mouse IgG. (*B*, *D*, *F*, and *H*) Actin organization, visualized by staining with rhodamine-phalloidin. Cells were fixed and stained at 10 min (*A* and *B*), 1 (*C* and *D*), 4 (*E* and *F*), and 24 h (*G* and *H*) after injection. Stress fibers disintegrate ~1 h (*D*) and regrow again in cells 24 h after injection. With the disruption of stress fibers, multiple lamellar lobes, with jagged margins, appear. BM1, which spreads rapidly throughout the cell after injection, collects in large patches in the rear parts of the cell after the breakdown of stress fibers and remains there for many hours (*G* and *H*). Bar, 10 μm.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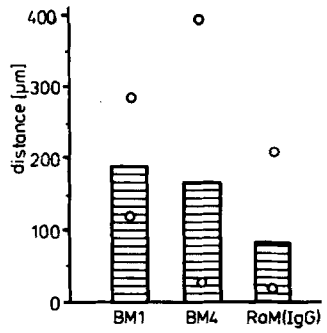

Figure 6. Increase in locomotory activity of chicken fibroblasts showing an altered morphology after injection with BM1 and BM4 (both at 8–9 mg/ml). (*RaM (IgG)*) Cells injected with rabbit anti-mouse antibody (26 mg/ml, controls). Ordinate: distance measured between the position of the nucleus of each cell before and 14 h after the injection. The bars represent average values from at least 30 cells scored. (*Open circles*) See legend to Fig. 4.

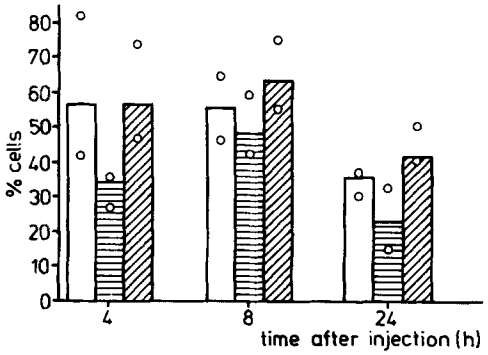

Figure 7. Number of cells that lost stress fibers and developed an abnormal morphology in response to the injection of BM1 (8 mg/ml, *open bars*), BM2 (9.3 mg/ml, *hatched bars*), or BM4 (9.2 mg/ml, *striped bars*). Cells at the 10th passage were injected. (*Open circles*) See legend to Fig. 4.

injected cells: in cultures of later passages, the proportion responding to antimyosin injection was higher than in cultures of younger cells (cf. Fig. 3 with Figs. 4 and 5). The actin cytoskeleton in noninjected controls of early and late passage cells showed no difference, and the nature of this "physiological factor" remains unknown.

The disruptive effect of antimyosins on stress fibers corroborates other observations that stress fibers, although composed of sarcomeric units, are highly dynamic structures that react sensitively to a variety of different agents (e.g., ATP-depleting substances, actin-capping, and actin-severing proteins; cf. references 13, 35, 36). The cleavage ring of dividing cells is also composed of sarcomeric units, and indeed, a polyclonal antimyosin has been shown before to interfere with its proper functioning during cytokinesis of starfish cells (29). In accordance with this result, we found that the mitotic index of BM1-injected cells was decreased: within a time period of 14 h, only 7% divided, as compared with 25% of the controls (Höner, B., and B. M. Jockusch, unpublished data). Thus, the effects of the injected antibodies support the theory on the role of myosin organized in sarcomere-like structures in nonmuscle cells.

However, our observations of an increase in cellular motility as a consequence of antimyosin injection were unexpected. The dynamic processes leading to lamellar spreading at the periphery of the injected cells were similar to controls. Microspikes and small ruffles formed rapidly, and thin membrane lobes erupted in close proximity to those structures (see 34 for a detailed analysis of the motility phenomena at the leading edge of normal chicken embryo fibroblasts). There were, however, two important differences. (*a*) The membraneous lobes protruding next to microspikes and ruffles seemed smaller and thinner than in controls, with ragged rather than smooth margins. (*b*) Membrane protrusions developed along the entire periphery of the cells, inducing gross shape changes. Such observations led to the impression that the antimyosin caused the cells to become more "fluid," having lost control over turgor or surface tension.

Since the antibodies employed had different effects on myosin filament formation in vitro, we had anticipated differential effects on injected cells. However, the differences observed were rather subtle. The antibodies collected in different areas of the cell (cf. Fig. 1 with Fig. 8), and BM2, which has the least drastic effect on myosin filament formation in vitro (Table I), seemed somewhat less potent after microinjection than BM1 and BM4 (Fig. 7). Much more conspicuous was the notion that all three antibodies were capable of changing actomyosin organization, cell shape, and locomotory activity. This implies that it is the antibody-induced decrease in functional myosin that triggers an increase in these events, rather than differences in myosin filament length or conformation.

The antibody-induced precipitation of myosin is bound to deprive the cell transiently of a large amount of this protein. Increase in membrane dynamics and locomotion may then be triggered either by small amounts of myosin not bound to the antibody, but released from stress fibers, or by other proteins capable of interacting with actin. The latter hypothesis is consistent with recent observations in *Dictyostelium discoideum*. Here, the reduction or even elimination of the conventional myosin by genetic methods yielded mutant cells which displayed ameboid movement and the development of pseudopodia and filopodia on solid surfaces. Concomitantly, cytokinesis was impaired, and, as a consequence, the cells grown in suspension became multinucleate (10, 25).

In *Dictyostelium* as well as in *Acanthamoeba*, proteins have been described that show an actin-activated ATPase activity and are thus probably mechano-enzymes of the myosin type, but without the ability to form bipolar filaments (cf. references 9, 30, 33). Since proteins of that type should have

Figure 5. Locomotory activity and shape change of cells during a period of 11 h after microinjection. (*Left*) Sequence of a cell injected with rabbit anti-mouse IgG (22 mg/ml). (*Right*) Sequence of a cell which received BM1 (8 mg/ml). The positions of both cells with respect to a reference point (*arrow*) at various time points during this period are emphasized in the drawings (bottom part of both panels). Bar, 10 µm.

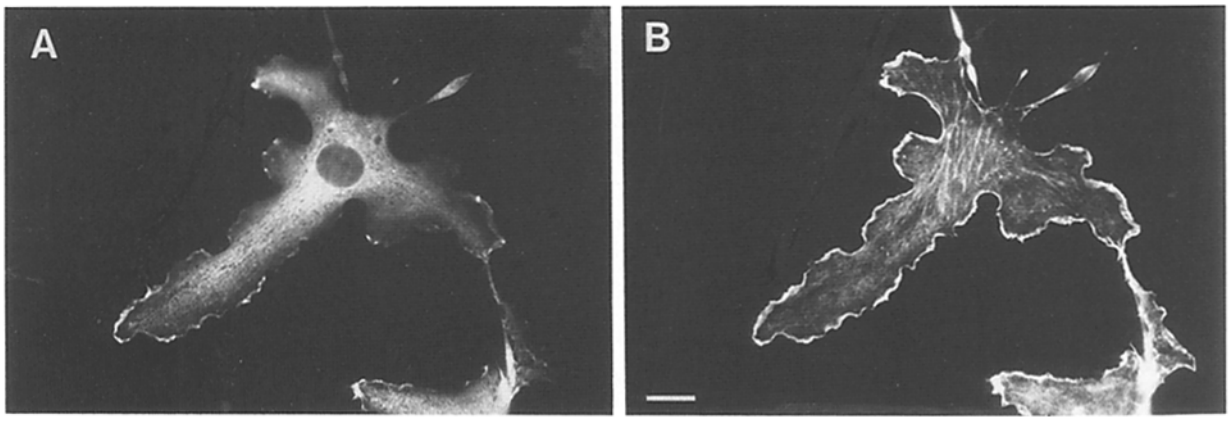

Figure 8. Distribution of BM4 and the actin cytoskeleton in a cell 24 h after BM4 injection (9.2 mg/ml). (*A*) Staining pattern seen with FITC rabbit anti-mouse IgG; (*B*) pattern seen with rhodamine-phalloidin. In contrast to BM1 (cf. Fig. 1 *G*), BM4 does not concentrate in patches at the rear end of the cell but remains spread throughout the cytoplasm. Bar, 10 μm.

remained unaltered in the mutants described above, one might speculate that these myosins may be involved in dynamic events at the plasma membrane leading to locomotion. Recently, evidence for similar proteins in higher eukaryotes, i.e., *Drosophila* (31), chicken (6–8), and cow (18) has been presented. It remains to be seen if indeed different members of the myosin superfamily can be functionally correlated with different types of cellular motility.

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References

1. Abercrombie, M. 1980. The crawling movement of metazoan cells. *Proc. R. Soc. Lond. B Biol. Sci.* 207:129–147.
2. Burridge, K. 1981. Are stress fibers contractile? *Nature (Lond.).* 294:691–692.
3. Citi, S., and J. Kendrick-Jones. 1987. Studies on the structure and conformation of brush border myosin using monoclonal antibodies. *Eur. J. Biochem.* 165:315–325.
4. Citi, S., and J. Kendrick-Jones. 1988. Brush border myosin filament assembly with monoclonal antibodies. *J. Muscle Res. Cell Motil.* 9:306–319.
5. Clarke, M., and J. A. Spudich. 1977. Nonmuscle contractile proteins: the role of actin and myosin in cell motility and shape determination. *Annu. Rev. Biochem.* 46:797–822.
6. Collins, J. H., and C. W. Borysenko. 1984. The 110,000-dalton actin- and calmodulin-binding protein from intestinal brush border is a myosin-like ATPase. *J. Biol. Chem.* 259:14128–14135.
7. Coluccio, L. M., and A. Bretscher. 1987. Calcium-regulated cooperative binding of the microvillar 110K-calmodulin complex to F-actin: formation of decorated filaments. *J. Cell Biol.* 105:325–333.
8. Conzelman, K. A., and M. S. Mooseker. 1987. The 110-kD protein–calmodulin complex of the intestinal microvillus is an actin-activated Mg ATPase. *J. Cell Biol.* 105:313–324.
9. Cote, G. P., J. P. Albanesi, T. Ueno, J. A. Hammer III, and E. D. Korn. 1985. Purification from *Dictyostelium discoideum* of a low-molecular weight myosin that resembles myosin I from *Acanthamoeba castellanii*. *J. Biol. Chem.* 250:4543–4546.
10. De Lozanne, A., and J. A. Spudich. 1987. Disruption of the *Dictyostelium* myosin heavy chain gene by homologous recombination. *Science (Wash. DC).* 236:1086–1091.
11. Elliot, G. F., and G. Offer. 1978. Shape and flexibility of the myosin molecule. *J. Mol. Biol.* 123:505–519.
12. Füchtbauer, A. 1984. Analyse des Mikrofilamentsystems tierischer zellen durch Mikroinjektion. Ph.D. thesis. University of Bielefeld, Bielefeld, FRG. 1–34.
13. Füchtbauer, A., B. M. Jockusch, H. Maruta, M. W. Kilimann, and G. Isenberg. 1983. Disruption of microfilament organization after injection of F-actin capping proteins into living tissue culture cells. *Nature (Lond.).* 304:361–364.
14. Fujiwara, K., and T. D. Pollard. 1976. Fluorescent antibody localization of myosin in the cytoplasm, cleavage furrow, and mitotic spindle of human cells. *J. Cell Biol.* 71:848–875.
15. Graessmann, M., and A. Graessmann. 1976. "Early" simian-virus-40 specific RNA contains information for tumor antigen formation and chromatin replication. *Proc. Natl. Acad. Sci. USA.* 73:366–370.
16. Haemmerli, G. 1985. Principles of cell motility and their morphologic manifestations. A comparative study of different cell classes moving on glass and in a tissue. *Exp. Biol. Med.* 10:98–117.
17. Höner, B., and B. M. Jockusch. 1988. Stress fiber dynamics as probed by antibodies against myosin. *Eur. J. Cell Biol.* In press.
18. Hoshimaru, M., and S. Nakanishi. 1987. Identification of a new type of mammalian myosin heavy chain by molecular cloning. *J. Biol. Chem.* 262:14625–14632.
19. Huxley, H. E. 1963. Electron microscopic studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* 7: 281–308.
20. Isenberg, G., P. C. Rathke, N. Hülsmann, W. W. Franke, and K. E. Wohlfarth-Bottermann. 1976. Cytoplasmic actomyosin fibrils in tissue culture cells. Direct proof of contractibility by visualization of ATP-induced contraction in fibrils isolated by laser microbeam dissection. *Cell Tissue Res.* 166:427–443.
21. Jockusch, B. M., A. Füchtbauer, Ch. Wiegand, and B. Höner. 1986. Probing the cytoskeleton by microinjection. *In* Cell and Molecular Biology of the Cytoskeleton. J. W. Shay, editor. Plenum Publishing Corp., New York. 1–40.
22. Deleted in proof.
23. Kendrick-Jones, J., R. Smith, and S. Citi. 1987. Regulation and organization of non-muscle myosins. *In* Progressive Zoology. Vol. 34. Nature and Function of Cytoskeletal Proteins in Motility and Transport. K. E. Wohlfarth-Bottermann, editor. Gustave Fischer Verlag, Stuttgart. 87–96.
24. Kiehart, D. P., I. Mabuchi, and S. Inoue. 1982. Evidence that myosin does not contribute to force production in chromosome movement. *J. Cell Biol.* 94:165–178.
25. Knecht, D. A., and W. F. Loomis. 1987. Antisense RNA inactivation of myosin heavy chain gene expression in *Dictyostelium discoideum*. *Science (Wash. DC).* 236:1081–1086.
26. Kreis, T. E., and W. Birchmeier. 1980. Stress fiber sarcomeres of fibroblasts are contractile. *Cell.* 22:555–561.
27. Langanger, G., M. Moeremans, G. Daneels, A. Sobieszek, M. De Brabander, and J. De Mey. 1986. The molecular organization of myosin in stress fibers of cultured cells. *J. Cell Biol.* 102:200–209.
28. Lowey, S., H. S. Slayter, A. G. Weeds, and G. Baker. 1969. Structure of the myosin molecule I. Subfragments of myosin by enzymic degradation. *J. Mol. Biol.* 42:1–29.
29. Mabuchi, I., and M. Okuno. 1987. The effect of myosin antibody on the division of starfish blastomeres. *J. Cell Biol.* 74:251–263.
30. Maruta, H., H. Gadasi, J. H. Collins, and E. D. Korn. 1979. Multiple forms of Acanthamoeba myosin-I. *J. Biol. Chem.* 254:3624–3630.
31. Montell, C., and G. M. Rubin. 1988. The Drosophila ninaC locus encodes two photoreceptor cell specific proteins with domains homologous to protein kinases and the myosin heavy chain head. *Cell.* 52:757–772.
32. Pollard, T. D., and R. R. Weihing. 1974. Actin and myosin and cell movement. *CRC Crit. Rev. Biochem.* 2:1–65.
33. Pollard, T. D., W. F. Stafford, and M. E. Porter. 1978. Characterization

of a second myosin from Acanthamoeba castellanii. *J. Biol. Chem.* 253:4798–4808.
34. Rinnerthaler, G., B. Geiger, and J. V. Small. 1988. Contact formation during fibroblast locomotion: involvement of membrane ruffles and microtubules. *J. Cell Biol.* 106:747–760.
35. Sanger, J. M., B. Mittal, A. Wegner, B. M. Jockusch, and J. W. Sanger. 1987. Differential response of stress fibers and myofibrils to gelsolin. *Eur. J. Cell Biol.* 43:421–428.
36. Sanger, J. W., J. M. Sanger, and B. M. Jockusch. 1983. Differential response of three types of actin filament bundles to depletion of cellular ATP levels. *Eur. J. Cell Biol.* 31:197–204.
37. Yumura, S., H. Mori, and Y. Fukui. 1984. Localization of actin and myosin for the study of amoeboid movement in *Dictyostelium* using improved immunofluorescence. *J. Cell Biol.* 99:894–899.