Probing the Role of Nonmuscle Tropomyosin Isoforms in Intracellular Granule Movement by Microinjection of Monoclonal Antibodies

Theresa E. Hegmann, Jenny Li-Chun Lin, and Jim Jung-Ching Lin
Department of Biology, University of Iowa, Iowa City, Iowa 52242

Abstract. Chicken embryo fibroblast (CEF) cells were microinjected with several different monoclonal antibodies that recognize certain nonmuscle isoforms of tropomyosin. Immediately after injection, cells were recorded with a time-lapse video imaging system; later analysis of the tapes revealed that particles in cells injected with one of these antibodies (CG1, specific for CEF tropomyosin isoforms 1 and 3) showed a dramatic decrease in instantaneous speed while moving, distance moved per saltation, and proportion of time spent in motion. Injection of Fab fragments of CG1 resulted in similar changes in the pattern of granule movement. This inhibition of granule movement by CG1 antibody was reversible; at 2.5 h after injection, granules in injected cells had already reached three-fourths of normal speed. The speed of granule movement in cells injected either with antibody specific for tropomyosin isoforms not present in CEF cells, or with CG1 antibody preabsorbed with tropomyosin, was not significantly different from the speed of granules in uninjected cells. When cells were injected with CG1 or Fab fragments of CG1, fixed, and counter-stained with rabbit antibodies to reveal the microtubule, microfilament, and intermediate filament systems, no obvious differences from the patterns normally seen in uninjected cells were observed. Examination of the ultrastructure of injected cells by EM confirmed the presence of apparently intact and normal microtubule, actin, and intermediate filament networks.

These experiments suggest that tropomyosin may play an important role in the movement of vesicles and organelles in the cell cytoplasm. Also, we have shown previously that the CG1 determinant can undergo a motility-dependent change in reactivity, that may be important for the regulatory function of nonmuscle tropomyosin (Hegmann, T. E., J. L.-C. Lin, and J. J.-C. Lin. 1988. J. Cell Biol. 106:385–393). Therefore, in addition to postulated microtubule-based motors, microfilaments may play a critical role in regulating granule movement in nonmuscle cells.

Many different types of movement can be observed in cells at the light microscope level, including translocation, cytoplasmic streaming, ruffling and blebbing, chromosome movement, cytokinesis, axonal transport, and saltatory movement of intracellular particles. In the particular case of saltatory motion, it has long been suspected that filamentous systems of some sort are involved (Rebhun, 1964), but a general mechanism for this process has not yet been described. It appears unlikely that intermediate filaments play an important role in particle movement, since several microinjection studies have shown that even after the collapse of vimentin or keratin filaments, particles in injected cells still demonstrate apparently normal saltatory movements (Gawlitta et al., 1981; Klymkowsky, 1981; Lin and Faramisco, 1981). It is generally believed that microtubules play the key role in saltatory motion of cytoplasmic particles (Allen et al., 1985; McNiven and Porter, 1984; Schnapp et al., 1985; Vale et al., 1986), but some convincing evidence for the participation of actin filaments also exists (Araki and Ogawa, 1987a; Euteneuer and Schlwa, 1984; Goldberg et al., 1980; Sheetz and Spudich, 1983; Adams and Pollard, 1986).

In this study, we present evidence for the involvement of tropomyosin in the saltatory movement of particles in chicken embryo fibroblast (CEF) cells. Tropomyosin is a rod-shaped protein that lies along actin filaments; in muscle cells, its role in the regulation of contraction has been well characterized (Ebashi et al., 1969; Lehman and Szent-Gyorgyi, 1975), but in nonmuscle cells, its function has not been completely determined. We have previously shown that CEF cells contain five isoforms (a, b, 1, 2, 3) of tropomyosin, as resolved in SDS-PAGE (Lin et al., 1984). In the experiments described here, four different monoclonal antibodies that recognize different combinations of tropomyosin isoforms have been microinjected into CEF cells. The tropomyosin binding properties of these antibodies are: CG3, all CEF isoforms; GGβ6, CEF isoforms a, b, 1, and 2; CGI CEF isoforms 1 and 3; and CH291, no CEF isoforms (Lin et al., 1984).

Abbreviations used in this paper: CEF, chicken embryo fibroblast.
In addition, two rabbit polyclonal antibodies that recognize all CEF tropomyosin isoforms were also used for microinjection (Lin et al., 1985). Of these six antibodies, only the monoclonal CGI and the two polyclonal antibodies significantly inhibited intracellular granule movement. Double-label immunofluorescence experiments demonstrated that this inhibition appeared to take place in the absence of disruption to the microtubule and intermediate filament networks.

**Materials and Methods**

**Cell Culture**

Primary cultures of fibroblast cells were prepared from the skin of 10-11-d-old chicken embryos by dissection and trypsinization, as described previously (Lin et al., 1984). Cells were cultured in DME with 10% FBS, and were kept in a humidified incubator at 37°C and 5% CO₂. Cells were used for microinjection and staining between the second and sixth passing.

**Antitropomyosin Antibodies**

The preparation and characterization of antitropomyosin monoclonal antibodies CGI, CG3, CG6, and CH291 were reported previously (Lin et al., 1985). Antibody specificity was checked by both protein immunoblot analysis and immunoprecipitation. For immunoprecipitation, [35S]methionine-labeled cells were lysed in buffer containing 50 mM Tris, pH 8.0, 165 mM NaCl, 0.1 mM EGTA, 1% Triton X-100, and 1 mM PMSF. Total cell extracts were prepared and were used for immunoprecipitation by CGI and CG6 as reported previously (Lin et al., 1988a). In the case of antibodies, CH291 and CGI (IgG1 class), the addition of second antibody was omitted. Monoclonal antibodies C9 and C21 against caldesmon were used as IgG2a controls (Lin et al., 1988b). Antibody CGI recognizes CEF tropomyosin isoforms 1 and 3, antibody CG3 recognizes all CEF isoforms (a, b, 1, 2, and 3), and antibody CG6 recognizes isoforms a, b, 1, and 2. Antibody CH291 does not recognize any CEF tropomyosin isoforms, but reacts with chicken cardiac and skeletal muscle isoforms. Methods for preparing and characterizing rabbit antisera against chicken gizzard or CEF tropomyosin have been described previously (Lin et al., 1988a).

**Production of Fab Fragments**

The preparation of Fab fragments from CGI antibody was carried out according to the method described by Stanworth and Turner (1978). Purified antibody was mixed with papain at a protein to enzyme weight ratio of 100:1. This mixture was incubated for 2-3 h at 37°C and then dialyzed against 10 mM sodium acetate buffer, pH 5.5. It was then passed over a CM-32 cellulose column and eluted with a linear sodium acetate gradient from 10 mM to 100 mM, at pH 5.5. SDS-PAGE was used to check the content of each fraction. Fractions enriched in Fab fragments were pooled and concentrated by 45% saturated ammonium sulfate. Finally, Fab fragments were separated from Fc and whole antibody by passing the pooled fractions through a Sephadex G-100 column equilibrated with 20 mM NaHPO₄, 150 mM NaCl, 1 mM EDTA, pH 7.0. In some preparations, the CM-32 cellulose column chromatography was omitted. The purified Fab fragments were checked by SDS-PAGE to estimate the degree of contamination by whole antibody, and by immunofluorescence microscopy to determine activity.

**Microinjection**

Antibodies for microinjection were purified as described (Lin et al., 1985) and then dialyzed against injection buffer (10 mM KH₂PO₄, 75 mM KCl, pH 7.2) overnight. For preabsorption experiments, an excess (0.2 nmol) of purified TM isoforms 1 and 3 was precomplexed and then dissolved in rhodamine-conjugated CGI antibody (0.05 nmol). This mixture was incubated for 30 min at 4°C, and then centrifuged at 12,000 g for 15 min before microinjection.

Glass microcapillary tubes were pulled into needles using a horizontal needle-pulling machine as described by Graessmann et al. (1980). Needles were mounted in a Leitz micromanipulator, and loaded with antibody (10 mg/ml for unconjugated antibody and 1 mg/ml for conjugated antibody) by back pressure. CEF cells were grown on glass coverslips in 35-mm tissue culture dishes, and were viewed with an inverted microscope (Diavert; E.

**Immunofluorescence Microscopy**

Cells were grown on glass coverslips and microinjected as described above. After either 30 min or 3 h of recovery time, cells were fixed in 3.7% formaldehyde in PBS (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 80 mM NaH₂PO₄, pH 7.3), and permeabilized in -20°C acetone, as described previously (Lin and Feramisco, 1981). The coverslips were then incubated for 30 min each in FITC-conjugated anti-mouse IgG (heavy and light chains), rabbit antisera to vimentin or tubulin, and finally TRITC-conjugated goat anti-rabbit IgG (heavy and light chains), with 30-min washes in two changes of PBS between each of these steps. After a last wash in PBS, coverslips were dipped in distilled water, and then mounted on glass slides with 15% gelvatol-20-30 (polyvinyl alcohol; Monsanto Polymers and Petrochemical Co., St. Louis, MO), 33% glycerol, and 0.1% sodium azide in PBS. To visualize actin filaments, injected cells were fixed and permeabilized as before, incubated for 30 min in FITC-conjugated goat anti-mouse IgG, washed in PBS, incubated in rhodamine-conjugated phalloidin (Molecular Probes Inc., Junction City, OR) for 20 min, dipped into PBS and then distilled water, and mounted. Cells were viewed with an epifluorescence photomicroscope III with a 63 x phase-contrast objective (Carl Zeiss Inc., Thornwood, NY). Phase-contrast pictures were taken on Technical Pan film (Eastman Kodak Co., Rochester, NY), and fluorescence pictures were taken on Tri-X film (Eastman Kodak Co.).

**Electron Microscopy**

Small circles were scratched on the inside bottom of 35-mm tissue culture dishes, and all cells within the circles were microinjected with CGI Fab fragments at 10 mg/ml. After 3 h, the cells were rinsed in PBS and then fixed in a mixture (1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0). Cells were rinsed in phosphate buffer and then postfixed for 30 min in 1% OsO₄ in 0.1 M phosphate buffer. Afterwards they were dehydrated in ethanol and embedded in Epon. Thin sections of the injected cells were cut with an ultracut E microtome (Reichert-Jung, Vienna). Sections were stained with uranyl acetate and lead citrate, and then viewed and photographed with an electron microscope (300; Philips Electronic Instruments, Mahwah, NJ).

**Time-Lapse Photography and Analysis of Particle Movement**

Cells grown on glass coverslips were injected with rhodamine-conjugated antibodies, as described above. The coverslips were then quickly transferred to a perfusion chamber (Berg and Block, 1984) and viewed with the epifluorescence microscope (Carl Zeiss Inc.). Injected cells were located by fluorescence, and the phase-contrast image was videotaped with a camera (Data/MTI 70; Nuivicon; Dage/MTI, Inc., Michigan City, IN) connected to a Panasonic time-lapse video cassette recorder. The videotape was played back at six times real speed.

Granule speeds were calculated by measuring the duration and length of single saltations. Measurements were taken for eight randomly chosen moving granules in each of at least 10 cells/treatment, chosen from 2-3 different cultures. The average distance moved in a single saltation was calculated by measuring 20 saltations in each of 4 cells/treatment. Saltation distances were measured by tracing granule movements on clear plastic overlays attached to the monitor screen. The proportion of time particles moved was calculated using a stop-watch precise to 0.2 s to measure the approximate amount of time individual granules spent in motion out of a 120-s time interval. For each treatment, 10 granules in each of 4 cells were picked at random by using a random-number table to choose x and y coordinates on a transparent grid attached to the monitor. In all of these cases, uninjected control
cells were chosen from the same fields as the injected cells to control for differences such as temperature, cell crowding, chemical microenvironment, and age of the culture.

Results

Inhibition of Intracellular Granule Movement by an Antitropomyosin Antibody

Previous results from experiments involving CG1 antibody have suggested that the antigenic determinant recognized by this antibody may be critical to the function of nonmuscle tropomyosin, and that this function may in some way be connected to cell motility (Hegmann et al., 1988). To further investigate these ideas, CG1 antibody and several other control antibodies were microinjected into CEF cells to determine whether antibody binding to nonmuscle tropomyosin would interfere with any normal biological function of the cell.

Fig. 1 shows a Western blot analysis of the four different rhodamine-conjugated monoclonal antibodies that were used in this study: CG1 and Fab fragments of CG1, against CEF tropomyosin isoforms 1 and 3; CG3, against isoforms a, b, 1, 2, and 3; CGβ6, against isoforms a, b, 1, and 2; and CH291, which recognizes tropomyosin isoforms from cardiac and skeletal muscle, but does not react with nonmuscle isoforms. Immunoprecipitation analysis of CG1 and CH291 antibodies in which the antigen was presented in relatively native form confirmed that CH291 apparently does not recognize any CEF tropomyosin isoforms (Fig. 2). A protein band with apparent molecular mass >200 kD, as well as minor protein bands at 68 and 45 kD were found in all immunoprecipitates and considered to be nonspecific bands. In our experience, a higher background is always obtained when immunoprecipitation is performed in the absence of ionic detergents, such as SDS. Two rhodamine-conjugated polyclonal antibodies (called R2 and R6) were also used for microinjection. Both of these antisera recognize all isoforms of CEF tropomyosin (Lin et al., 1988a).
After antibody injection, treated cells were located by their fluorescence, and the phase-contrast image of the live cells was videotaped for later analysis. Microinjection did not appear to change normal cell morphology significantly except for a transient retraction that was also observed after injection of buffer; the only clearly identifiable effect of antitropomyosin antibody injection that we have found so far is the inhibition of intracellular granule movement in the case of CG1 monoclonal antibody and both polyclonal antibodies.

Table I summarizes the results of the first portion of the study. Three characteristics of granule movement were measured: instantaneous speed, proportion of time moving in 120 s, and distance moved in a single saltation (between pauses). The purpose of these measurements was not to provide a detailed description of organelle movement; this has already been done by others (for example, Hayden et al., 1983; Herman and Albertini, 1984). Rather, our goal was to try to elucidate something about the mechanism behind granule movement by comparing the movement of particles in injected and uninjected cells. The mean instantaneous speed of moving granules in uninjected cells was 20.8 μm/min. Cells injected with CG3, CGβ6, or CH291 had mean granule speeds that did not differ significantly from uninjected cells. Cells injected with CG1 antibody, however, showed a dramatic decrease in instantaneous granule speed to ~4.9 μm/min with the whole antibody and 2.9 μm/min with Fab fragments of CG1. This effect was abolished by preabsorbing CG1 with purified tropomyosin isoforms 1 and 3 before microinjection. To control for the possibility that the decrease in granule speed after injection of CG1 could be due to a cross-linking effect, Fab fragments of CG1 were also used for microinjection. These fragments appeared to have essentially the same effect as microinjection of the whole antibody (Table I). Injection of both rabbit antisera also resulted in a significant decrease in granule speed, but of differing magnitudes: R2 essentially stopped all movement, while R6 cut the speed of intracellular granule movement by about two-thirds (Table I).

The decrease in granule speed observed after injection of CG1 antibody was not permanent, and did not appear to be lethal to the cells. Beginning ~1 h after injection, the intracellular granules gradually resumed normal saltatory motions, so that by 2.5 h after injection they were moving on average approximately three quarters of normal speed with no significant difference in the average length of saltations (Fig. 3). Fig. 4 provides a method for visualizing the effects of antitropomyosin antibody injection on saltatory movement of cytoplasmic particles. Granules in cells injected with CG1 (Fig. 4, A and B) show very little displacement over a 150-s time period, while the granules in the cell injected with CG1 preabsorbed with tropomyosin (Fig. 4, C and D), and in the cell injected with CGβ6 (Fig. 4, E and F) display a pattern of movement indistinguishable from those in the uninjected cells.

Along with the drastic decrease in speed, the general pattern of saltatory movements also changed dramatically in cells injected with CG1 or the rabbit polyclonals, R2 and R6. The mean distance moved in a single saltation decreased

![Figure 3](image-url)
Figure 4. Effects of antitropomyosin CG1 antibody on intracellular granule movements. Video images (camera; DAGE/MTI 70; Nuvicon) of uninjected control and antibody-injected CEF cells are depicted in B, D, and F. Actual tracings of granule movements from videotaped live CEF cells are illustrated in A (CGI-injected), C (preabsorbed CGI-injected), and E (CGβ6-injected). Tracings were made over a period of 2.5 min, with 0.1-s frame intervals. The arrows in B, D, and F point to the injected cells. Long, linear excursions are characteristic of granule movements in uninjected control cells, CGβ6-injected cells, and cells injected with CGI antibody preabsorbed with purified CEF tropomyosin. On the contrary, granules in CGI-injected cells exhibit very little movement within the 2.5-min time period. Bar, 10 μm.

from 3.4 μm in uninjected cells to ~1 μm in cells injected with CGI, CGI Fab, R2, or R6 antibodies. In addition, the particles in these cells tended to spend a much smaller proportion of time in motion: only 2–6%, as opposed to ~36% in control cells and cells injected with CG3, or CGβ6 (Table I). In cells injected with CH291, the mean speed, proportion of time in motion and distance moved by particles are slightly smaller than that in control uninjected cells. However, these differences are not statistically significant (t test).
Tropomyosin Localization in Injected Cells

An actin binding assay and immunofluorescence studies were used in an attempt to determine whether the injection of antitropomyosin antibodies had an effect on tropomyosin localization in the cell. None of the four monoclonal antibodies used for injection showed a significant ability to interfere with the ability of CEF tropomyosins to bind to actin filaments in vitro binding assays (Fig. 5). As a positive control, CH1 antibody at 1 mg/ml was able to significantly interfere with the binding of skeletal tropomyosin to actin filaments under the same binding conditions. Although CG6 antibody at high concentration (4 mg/ml) had a slight effect on the binding of CEF tropomyosin to actin filaments, this effect could not be detected at concentrations lower than 3 mg/ml. Furthermore, injection of CG1 antibody at 1 mg/ml was sufficient to cause a significant inhibition of granule movement. Thus, these results suggest that the antibodies used in this study do not act to strip tropomyosin molecules from actin filaments.

Indirect double-label immunofluorescence experiments confirmed the results of the actin-binding assays. When CEF cells were injected with CG1 antibody at high concentration (20 mg/ml), fixed, and stained with rabbit antitropomyosin antibody (R2), the in vivo localization of tropomyosin did not appear to be disrupted significantly by the presence of CG1 antibody (Fig. 6). Apparently, the inhibition of granule movement in CEF cells after injection of CG1 antibody is not because of dissociation of tropomyosin molecules from actin filaments.

Microtubule, Microfilament, and Intermediate Filament Networks in Cells Injected with CG1

Saltatory movements of intracellular particles have long been associated with the cytoskeletal elements in the cell, so it might be expected that inhibition of granule movement could be related to an alteration or rearrangement in one or more of the cell's filamentous networks. To examine such an effect, CEF cells injected with mouse monoclonal CG1 antitropomyosin antibodies were double-labeled with rabbit antibodies against vimentin or tubulin, or else stained with rhodamine-conjugated phalloidin to label actin filaments (Fig. 7).

Microinjection of CG1 Fab fragments does not appear to have any disruptive effects on the microtubule, microfilament, or intermediate filament networks in these cells. Similarly, cells injected with whole CG1 antibody or CH291 antibody did not show any differences from control cells at the light microscope level. These experiments were repeated with a 3-h (rather than 30-min) recovery time after injection, with identical results (Fig. 8). However, it should be noted that there appears to be more CG1 antibody associated with stress fibers in cells that have recovered for 3 h than in cells that were fixed only 30 min after injection. With more time after injection, the antigenic determinants of the tropomyosin molecules may become more available for CG1 antibody.

CEF cells microinjected with Fab fragments of CG1 were also fixed for 20 min in 1% buffered glutaraldehyde and processed for transmission electron microscopy. Fig. 9 shows that intact intermediate filaments, microtubules, and microfilament bundles were visible at the ultrastructural level.

Discussion

Subcellular organelles and granules exist within a three-dimensional cytoskeletal network of actin filaments, microtubules, and intermediate filaments, and have been shown to associate with many of these and associated proteins, such as tubulin (Pratt, 1986), actin (Burridge and Phillips, 1975; Mehrabian et al., 1984), myosin (Burridge and Phillips, 1975), MAPS (Gilbert and Sloboda, 1986), and caldesmon (Bergoyne et al., 1986). The cytoskeleton not only defines the
Figure 6. Indirect immunofluorescence study on the localization of tropomyosin in CGI antibody-injected cells. CEF cells were microinjected with CGI monoclonal antibody and 30 min after injection were fixed and permeabilized. They were then incubated with rabbit antitropomyosin antibody (R2) and subsequently with a mixture of rhodamine-conjugated goat anti-rabbit IgG and fluorescein-conjugated goat anti-mouse IgG. (A) Injected cells viewed selectively for fluorescein fluorescence, to allow the microinjected mouse antibody to be visualized. (B) Same field seen in A except they are viewed selectively for rhodamine fluorescence, to allow the distribution of tropomyosin to be visualized. (C) Phase-contrast micrograph. The arrow points to the injected cell. Bar, 10 μm.

shape of a cell, but is also believed to be involved in diverse cellular processes. Locomotion (Clarke and Spudich, 1977; Gotlieb et al., 1983; Singer and Kupfer, 1986; Wehland and Willingham, 1983), endocytosis (Allison, 1973; Silverstein et al., 1977), secretion (Allison, 1973), organelle arrangement and movement (Araki and Ogawa, 1987b; Dabora and Sheetz, 1988; Freed and Lebowitz, 1970; Goldberg et al., 1980; Lee and Chen, 1988; Matteoni and Kreis, 1987; Porter, 1973; Schliwa, 1984; Terasaki et al., 1986; Wang and Goldman, 1978), cytoplasmic streaming (Rebhun, 1972; Schliwa, 1984), and mitosis (Gorbisky et al., 1987; Mitchison, 1986) have all been linked to various cytoskeletal proteins. Given these observations, it is natural to suspect that at least some components of the cytoskeleton play a critical role in forming a pathway for, or providing the force for, saltatory movement of subcellular particles.

Microtubules have been linked with saltatory motion of particles in the cytoplasm by many different investigators. Some of the lines of evidence include the existence of cross bridges between microtubules and membrane-bound organelles (Hirokawa, 1982), the observation that in many cell types the occurrence and directionality of saltatory movements are highly correlated with the presence and positioning of cytoplasmic microtubules (Freed and Lebowitz, 1970; Murphy and Tilney, 1974), and experiments in several different systems that have demonstrated that saltatory motion stops in the presence of microtubule inhibitors such as colchicine, vinblastine, and podophyllotoxin (Murphy and Tilney, 1974; Wang and Goldman, 1978). Observations that cytoplasmic organelles in keratocytes (Hayden, et al., 1983) and in the squid giant axon (Allen et al., 1985; Schnapp et al., 1985) appear to travel along single microtubules served to spur a search for the motor responsible for generating such movement. Development of in vitro assays for microtubule-dependent motility made possible the identification and purification of a soluble plus-end directed motor protein, kinesin (Vale et al., 1985). Later, MAP IC was identified as a cytoplasmic dynein analogue with microtubule-activated ATPase activity and minus-end directed microtubule translocating activity (Paschal et al., 1987). Thus, there is a great deal of evidence for the involvement of microtubules in at least some cases of cytoplasmic particle transport.

The involvement of actin filaments in intracellular granule movement has also been investigated in many different systems, with varying results. However, some evidence does exist to suggest that actin plays an important role. Both isolated chromaffin granules and lysosomal membranes have been demonstrated to interact with actin filaments in vitro (Araki and Ogawa, 1987b; Fowler and Pollard, 1982; Mehrabian et al., 1984), and chromaffin secretory granules have been shown to associate with actin-binding proteins such as caldesmon (Bergoyne et al., 1986). In addition, cytochalasins, known to destabilize actin filaments, have been shown to inhibit lysosomal movement in macrophages (Araki and Ogawa, 1987b). It has also been reported that microinjection of DNase I, which binds to and depolymerizes actin, inhibits fast axonal transport (Goldberg et al., 1980; Isenberg, 1980), and that gelsolin in the presence of micromolar Ca** inhibits the movement of membranous organelles in isolated axoplasm (Brady et al., 1984). In the case of fast axonal transport, it has even been suggested by some investigators that microtubules may not be required, since transport has been observed to continue at control levels in nerve axons in which the microtubules have been depolymerized by prein-
Figure 7. Indirect double-label immunofluorescence of CGI Fab fragment-injected cells. CEF cells were microinjected with Fab fragments of CGI monoclonal antibody and, 30 min after injection, were fixed and permeabilized. They were then incubated first with rhodamine-conjugated phalloidin (A–C), rabbit antitubulin antibody (D–F), or rabbit antivimentin antibody (G–I), and subsequently with a mixture of rhodamine-conjugated goat anti-rabbit IgG and fluorescein-conjugated goat anti-mouse IgG. (C, F, and I) phase-contrast micrographs. (A and D, G) Injected cells viewed selectively for fluorescein fluorescence, to allow the microinjected mouse antibody to be visualized. (B, E, and H) Same fields seen in A, D, and G, respectively, except they are viewed selectively for rhodamine fluorescence, to allow the distribution of actin (B), tubulin (E), and vimentin (H) to be visualized. Note that apparently normal distributions of microfilament bundles, microtubules, and intermediate filaments are observed in the CGI-injected cells. Bar, 10 μm.

cubation in buffer containing 75 mM Ca++ (Brady et al., 1980).

Apparently, microtubules and actin filaments are somehow involved in particle transport in many different cell types. But the exact role of these different cytoskeletal components remains a mystery: do they provide the motor, form a scaffold, define low viscosity channels through the cytoplasm, or act through some other mechanism yet to be proposed?

In this report, we have presented evidence that nonmuscle
Figure 8. Indirect double-label immunofluorescence of CGI Fab fragment-injected cells 3 h after injection. Staining protocol was identical to that of Fig. 4. (A, D, and G) Injected cells viewed selectively for fluorescein, to allow the microinjected mouse antibody to be visualized. (B, E, and H) Same fields seen in A, D, and G, respectively, except they are viewed selectively for rhodamine fluorescence, to allow the distribution of actin (B), tubulin (E), and vimentin (H) to be visualized. (C, F, and I) phase-contrast micrographs. Microfilament bundles, microtubules, and intermediate filaments still appear to be undisturbed. Bar, 10 μm.
Figure 9. Electron micrograph of CEF cells microinjected with CGI monoclonal antibody. Cells were fixed for 20 min in 1% glutaraldehyde. 
mf, microfilaments; mt, microtubules; if, intermediate filaments. Bars, 200 μm.
tropomyosin, an actin-binding protein, may play an important role in motility at the subcellular level. This conclusion is supported by previous studies that have led us to believe that the CGI antigenic determinant is important to the function of nonmuscle tropomyosin. For example, we have shown earlier that CGI preferentially stains the stress fibers of CEF cells that display a motile morphology (Hegmann et al., 1988). In addition, the CGI epitope contains a cysteine residue that may be analogous to the cyst-190 residue in skeletal tropomyosin which has been shown to participate in a conformational change in the tropomyosin molecule. This ability to undergo a conformational change is thought to facilitate the regulatory role of tropomyosin in muscle contraction (Lerher et al., 1981). Significantly, chemical modifications of nonmuscle tropomyosin at cysteine residues by performic acid oxidation or 5,5'-dithiobis-(2-nitrobenzoic acid) cross-linking drastically change the ability of CGI to recognize tropomyosin isoforms 1 and 3 by immunoblotting (Hegmann et al., 1988).

In the present study, inhibition of intracellular granule movement after injection of CGI antibody appeared to be specifically because of the tropomyosin-binding capability of this antibody, since preabsorption with purified tropomyosin isoforms abolished the effect, and in vitro binding assays have shown that CGI does not have the ability to interfere with the binding of tropomyosin to actin filaments. Furthermore, inhibition of granule movement was observed only after injection of CGI monoclonal antibody or rabbit polyclonal antibodies against tropomyosin; other antitropomyosin monoclonal antibodies had no effect. This result suggests that the particular epitope recognized by CGI antibody is critical to the proper functioning of nonmuscle tropomyosin in the regulation of intracellular motility.

The results presented here certainly do not rule out the participation of microtubules in intracellular granule movement. Rather, they provide strong evidence that at least some subset of intracellular motility requires actin filaments and their associated protein, tropomyosin, in addition to microtubules. This idea is further supported by preliminary results indicating that injection of a certain subset of monoclonal antibodies against caldesmon (a Ca++/calmodulin-binding and actin-binding protein found in smooth muscle and nonmuscle cells; Bretscher, 1986; Sobue et al., 1981; Lin et al., 1988b) also results in reversible inhibition of intracellular granule movement in CEF cells. Perhaps further research along these lines will help to elucidate the role of actin and actin-binding proteins in the regulation of motility at the cellular and subcellular levels.

We would like to thank Dr. Eugenia Wang for the use of rabbit antivimentin antibody.

This work was supported in part by grants HD18577 and GM40580 from the National Institutes of Health and by grants from the Muscular Dystrophy Association and the Pew Memorial Trust. Dr. J. J.-C. Lin is a recipient of a Pew Scholarship in Biomedical Sciences from the Pew Memorial Trust.

Received for publication 7 October 1988 and in revised form 12 May 1989.

References
Lin, J. J. C., and J. R. Feramisco. 1981. Disruption of the in vivo distribution...


