Motility-dependence of the Heterogenous Staining of Culture Cells by a Monoclonal Anti-tropomyosin Antibody

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Abstract. A monoclonal antibody (CG1) which recognizes tropomyosin isoforms 1 and 3 of chicken embryo fibroblasts was used to detect what is a motility-dependent change in the availability of the antigenic determinant in tropomyosin molecules along microfilaments. Immunofluorescence microscopy with this antibody revealed a heterogenous staining pattern among chicken embryo fibroblasts cells such that a population (17%) of cells showed only background staining. Stress fibers in about half the population of the cells stained weakly with this antibody, while the stress fibers in another population of cells (35%) showed very strong staining. After glycerination or cytochalasin B treatment, all of the cells became positive in reaction to CG1 antibody, suggesting that the antigenic determinant was present in every cell. On the other hand, all of the cells after brief nonionic detergent treatment became negative to CG1 antibody. The CG1 staining pattern was not significantly changed in cells at differ-

ent stages after release from colcemid blockage, nor was a brief treatment of cells with buffer containing 2 M urea, mild trypsin, chymotrypsin, or V.8 protease effective in changing the reactivity. However, most of the cells with a morphology typical of movement, and all of the contracted, glycerinated cells were strongly positive to CG1 antibody. These results suggest that the unmasking of the CG1 determinant may be motility-dependent. Immunoblot analysis showed that forced modification on the cysteine residue of tropomyosin molecules, caused either by performic acid oxidation or by disulfide cross-linking with the chemical 5,5'-dithiobis (2-nitrobenzoate), results in drastic changes in the reactivity of the different isoforms to CG1 antibody. These results indicate that the cysteine residue is involved in the CG1 determinant. The motility-dependent unmasking of this determinant may suggest an important role for nonmuscle tropomyosin in regulating cell motility.

TROPOMYOSIN is a dimeric coiled-coil protein that binds along the length of actin filaments. It is associated with the thin filaments of muscle cells and the microfilaments of nonmuscle cells (5, 28, 33). We have previously shown that chicken embryo fibroblasts (CEF)[1] contain at least five isoforms of tropomyosin (a, b, 1, 2, and 3), identified as such by their different apparent molecular masses after separation by SDS–PAGE, but similar biochemical properties, such as resistance to heat and organic solvents, the ability to bind to F-actin filaments, and the lack of proline and tryptophan (25, 26). It is not known whether the different isoforms have different localizations and/or functions within CEF cells. However, such a situation is suggested by the fact that transformation of CEF cells by Rous sarcoma virus results in an altered pattern of expression of tropomyosin isoforms (25).

Monoclonal antibodies have shown great promise as a tool for the study of tropomyosin isoforms. Many monoclonals have been developed against CEF tropomyosins (23), one of

which has been shown by immunoblotting to react only with CEF tropomyosin isoforms 1 and 3. This antibody, called CG1, displays an interesting and unusual staining pattern. A population of CEF cells (∼17%) show only background staining. In the present study, we have shown that nonstaining cells can be experimentally induced to stain positively either by cytochalasin B treatment to disrupt microfilament bundles, or by glycerol extraction. This result indicates that antigens recognized by CG1 antibody do exist in every cell. The availability of the CG1 antigenic determinant correlates well with the motile morphology. Furthermore, we have shown that the cysteine residue of tropomyosin is involved in forming the antigenic determinant.

Materials and Methods

Cell Culture

Primary CEF cell cultures were prepared by dissection and trypsin digestion of the skin of 10-d-old chick embryos as described previously (26). Cells were maintained in DME containing 10% FCS and incubated at 37°C in a humidified chamber with 5% CO_2 and 95% air. Cells were used after the second, third, and fourth passagings.

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1. *Abbreviations used in this paper:* CEF, chicken embryo fibroblasts; DTNB, 5.5'-dithiobis (2-nitrobenzoate).

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Antitropomyosin Antibodies

The preparation and characterization of anti-tropomyosin monoclonal antibodies CG1, CGβ6, and CG3 were reported previously (23). The antibody CG1 recognizes isoforms 1 and 3 of CEF tropomyosin, the antibody CG3 reacts with all isoforms (a, b, 1, 2, and 3), and the antibody CGβ6 reacts with isoforms a, b, 1 and 2. Methods for preparing and characterizing rabbit antiserum against chicken gizzard tropomyosin were described in a previous report (32). This antiserum recognizes all isoforms of CEF tropomyosin.

Indirect Immunofluorescence

For immunofluorescence microscopy, cells were grown on 12 mm round glass coverslips to various densities (from 1 d after plating to confluency) before use. The standard procedure for indirect single-label or double-label immunofluorescence was performed as described previously (22), including 3.7% formaldehyde fixation and cold acetone permeabilization.

Several variations of this standard procedure were also performed. (a) Urea, chymotrypsin, trypsin, and V.8 treatment: after acetone extraction, cells were exposed to either 2 M urea or 20 µg/ml protease in PBS (137 mM NaCl, 27 mM KCl, 1.5 mM KH₂ PO₄, 8 mM Na₂HPO₄, pH 7.3) for 30 s followed by washes in PBS and application of the primary antibodies (9). (b) Nonionic detergent extraction: cells were extracted for 2 min in various concentrations (0.05, 0.1, 0.2, and 0.5%) of Triton X-100 or Nonidet P-40 in Tris buffer (10 mM TrisCl, pH 7.5, 150 mM NaCl, 1 mM MgCl₂) followed by a PBS rinse and then the standard fixation procedure. The detergent treatment was also performed after formaldehyde fixation, rather than before. (c) Colcemid treatment: cells grown on glass coverslips were treated with a low dose of colcemid (0.06 µg/ml) for 12 h. At various periods of time (15 min, 3, 13, and 18 h) after removal of the colcemid, coverslips were fixed and stained by the standard procedure. (d) Cytochalasin B treatment: cells grown on glass coverslips were treated with 10 µg/ml of cytochalasin B. After 30 min the cells arborized, and then the medium was replaced with fresh medium. At 0, 10, and 25 min after the reversal, cells were fixed and stained by the standard procedure. (e) Glycerol-extracted cell models: the cell models used for inducing cell contraction were prepared exactly as described by Kreis and Birchmeier (15, 16). Briefly, cells grown on glass coverslips were lysed with buffer containing 50% glycerol, 50 mM KCl, 5 mM EDTA, 10 mM Tris, pH 7.5, at 4°C for 2 h and then rinsed in contraction buffer (30 mM KCl, 5 mM MgCl₂, 10 mM CaCl₂, and 10 mM Tris, pH 7.5). These cells were then induced to contract by the addition of ATP and MgCl₂ in contraction buffer, at a final concentration of 0.2 mM each. At various periods of time (2, 10, and 20 min), the contracted cells were fixed and double-labeled by the standard procedure with CG1 monoclonal antibody and rabbit anti-tropomyosin antiserum. In another variation, two

100-mm dishes of CEF cells at confluence were extracted with 2 ml glycerol extraction buffer each. The extract was collected and dialyzed against contraction buffer, and then glycerol-extracted models of cells grown on glass coverslips were incubated in the dialyzed extract for 30 min at room temperature. Finally, the cells were fixed and stained for immunofluorescence according to the standard procedure described previously. The stained cells were observed and photographed using a Zeiss epifluorescence photomicroscope III with a Zeiss 63× oil-phase 3 lens as described previously by Blose (3).

Performic Acid Oxidation and Protein Immunoblotting

The CEF tropomyosin isoforms were prepared as described previously (25). The individual isoforms were further purified by electroelution of the appropriate gel bands from preparative SDS-polyacrylamide gels (23). After the removal of SDS by 80% cold acetone, the individual isoforms were allowed to renature and were used for performic acid oxidation and protein immunoblotting.

Performic acid oxidation of the individual isoforms was carried out according to Hirs (14). Briefly, the lyophilized tropomyosin isoforms were dissolved in 0.2 ml performic acid reagent containing 1 vol of 30% hydrogen peroxide and 9 vol of 88% formic acid. The mixture was left in an ice bath for 2 h, and then the excess reagent was removed by repeated lyophilization.

Protein immunoblotting was performed as described by Towbin et al. (35). The individual isoforms and their oxidized counterparts were first separated on 12.5% SDS-PAGE (23) and then electrophoretically transferred to strips of nitrocellulose paper. One blot was stained with Amido black to reveal proteins, and a replicate blot was immunoreacted with CG1 antibody as described previously (23).

Tropomyosin Isoform Dimer Formation

The chemical 5.5'-dithiobis (2-nitrobenzoate), or DTNB, was used to form intramolecular cross-links between opposing cysteine residues of the purified tropomyosins as described previously (25). Briefly, the tropomyosin (0.2-0.5 mg/ml) was reacted with 10 mM DTNB for 30 min at room temperature, and then the samples were solubilized in SDS-gel sample buffer without reducing agent dithiothreitol (DTT), and run on a 12.5% SDS-PAGE. To identify which isoforms formed dimers, the gel track was cut out and placed on top of a new SDS-polyacrylamide slab gel. This second dimension was run under reducing conditions by overlaying the gel track with sample buffer containing 100 mM DTT and 1% agarose.

Results

The monoclonal antibody CG1 recognizes a determinant present in CEF tropomyosin isoforms 1 and 3, but not isoform 2 (23). Immunofluorescence microscopy with this antibody revealed stress fiber staining only on a subpopulation of CEF cells (Table I and Fig. 10 in reference 23). Scoring the cells by fluorescent intensity, we found that ∼17% of the cells showed no staining, another 35% showed strong staining, and about half the population of the cells showed weak staining (control in Table I). This mixed staining pattern was not changed at a 100-fold higher concentration of CG1 antibody. An identical result was also obtained when the standard fixation procedure was replaced by cold methanol (data not shown).

We have noted that those cells with strong staining frequently possess an identifiable leading lamella and a retraction tail, suggesting that they were actively moving immediately before they were fixed (1). Spindle-shaped cells and ameboid-shaped cells with pseudopod-like extensions also tend to have a much larger strong-staining population than do cells that are not in any of these categories (Table II). These three classes of cells (wedge-shaped, spindle-shaped, and ameboid-like) have been identified as actively motile by Herman et al. (13), using time-lapse videotaping. Thus, our observations suggest that the positive-staining state of the

Table I. Populations of Control and Treated CEF Cells Stained with CG1 Monoclonal Antibody for Immunofluorescence Microscopy

Treatment	Total number of cells scored	Degree of CG1 staining		
		Negative	Weak	Strong
		%	%	%
Control	853	16.8	48.7	34.6
Confluent	500	94.4	–	5.6
Spreading*	181	4.4	63	32.6
Cytochalasin	67	0	0	100
Glycerol	70	0	0	100
Contracted‡	100	0	0	100
Triton X-100	45	100	0	0
Trypsin	183	14.8	47.5	37.7
Chymotrypsin	177	15.8	49.7	34.5
V.8 protease	179	12.8	51.9	35.2
Urea	165	16.4	52.1	31.5

Control and treated CEF cells were stained with CG1 antibody for indirect immunofluorescence microscopy. The cells were then scored for intensity of CG1 staining.
* Cells were passaged 2 h before fixation and processing for immunofluorescence microscopy.
‡ Cells were fixed 10 min after addition of 0.2 mM Mg-ATP to induce contraction.

Table II. Comparison of Cell Shape and Degree of CG1 Staining

Cell shape*	Movement*	Total number of cells scored	Degree of CG1 staining		
			Negative	Weak	Strong
			%	%	%
Wedge-shaped, with leading lamella and retraction tail	+ membrane ruffling	194	6.2	42.3	51.5
Spindle-shaped	+ + + translocating (.5–3.5 μm/min)	102	2	29.4	68.6
Ameboid-like	+ + + translocating (.5–3.5 μm/min)	102	4.9	24.5	70.6
Others	Not characterized	455	27.3	61.1	11.6
Total		853	16.8	48.7	34.6

CEF cells were stained with CG1 for indirect immunofluorescence microscopy. The cells were then scored for degree of staining.
* These cell shape classifications and the information on degree and nature of cell motility were taken directly from Herman et al., 1981 (13).

CG1 determinant may be related to cell motility. This idea is also supported by the CG1 staining pattern displayed by cells that are spreading after replating, and by cells that have reached confluence. As can be seen from the data in Table I, a smaller proportion of spreading cells do not stain with CG1, as would be expected of cells that are assumed to be in motion. On the other hand, cells that have reached confluence and are being held in check by contact inhibition show a much larger population of negative cells: 94% as compared with 17% in the control group. Therefore, the availability of the CG1 determinant may be motility-dependent.

At least two possible explanations could account for the heterogenous staining of CEF cells by CG1 antibody. First, the unstained cells may contain no isoforms 1 and 3. This might be due to either the cell cycle–dependent expression of these isoforms or the contamination of our CEF preparations by other cell types. The second possible explanation is that in unstained cells, the CG1 determinant is inaccessible due either to the presence of masking proteins or to the specific conformation of tropomyosin molecules on the actin microfilaments. To distinguish between these possibilities, we have examined various preparative conditions which might perturb the organization of the microfilaments or unmask the determinant.

When cells were treated with cytochalasin B, which is known to disrupt microfilament bundles (10), CG1 antibody stained all cells (Fig. 1 B, Table I). This suggested that all cells in our preparation contained the antigens recognized by CG1 antibody. After the drug was washed away, the cells gradually reverted to the normal heterogenous CG1 staining pattern, and in addition regained their normal morphology (Fig. 1 D). In another attempt to determine whether CEF cells contain only certain isoforms of non-muscle tropomyosin, we labeled single cells with [35S]methionine and then ran two-dimensional gels. In each of five cases, the cell contained all tropomyosin isoforms (data not shown).

Glycerol-extracted cell models (16) and cells contracted after the addition of Mg++ and ATP also showed uniformly strong staining by CG1 antibody (Figs. 2 and 3). These results further argued against the possibility that some cells in our preparations contained no antigens recognized by CG1

antibody, and suggested that glycerol extraction may unmask the antigenic determinant either by removing masking proteins from the tropomyosin molecule or by inducing a local conformational change in tropomyosin. When the glycerol extract of CEF cells was dialyzed against contraction buffer and then added back to glycerol-extracted cell models, the heterogenous CG1 staining pattern did not reappear. The same results were also obtained with the add back experiment using a 10-fold higher concentration of glycerol extract. Therefore, this result does not support the possibility that glycerol extraction reveals the determinant by removing masking proteins.

In an attempt to rule out the possibility that the heterogenous staining by CG1 antibody was due to interphase cells at different stages of the cell cycle, cells partially synchronized by colcemid blockade were processed for immunofluorescence microscopy. Cells stained with CG1 antibody at different time intervals after the removal of colcemid showed no significant difference from control cells in the proportions of stained and unstained cells (data not shown). These results suggest that the heterogenous staining pattern exhibited by CG1 antibody does not result from the presence of CEF cells at different stages in the cell cycle.

The next challenge was to distinguish between the presence of a masking protein and a conformational change in the tropomyosin molecule. In an attempt to extract any masking protein, we treated the cells briefly with 2 M urea or mild trypsin, chymotrypsin, or V.8 protease solution and then stained them for immunofluorescence microscopy, according to the method of Franke (9). As can be seen in Table I, the treatments with 2 M urea or trypsin appeared to damage the cells somewhat, but the CG1 staining pattern was not altered in either case. Similar results were obtained with the other proteases. Again, these results do not support the possibility that a removable masking protein prevents the determinant from recognition.

Nonionic detergent extraction of the cells before staining for immunofluorescence changed the standard CG1 staining pattern greatly but did not affect staining by other monoclonal and polyclonal antibodies against tropomyosin. After a brief extraction with various concentrations of Triton X-100,
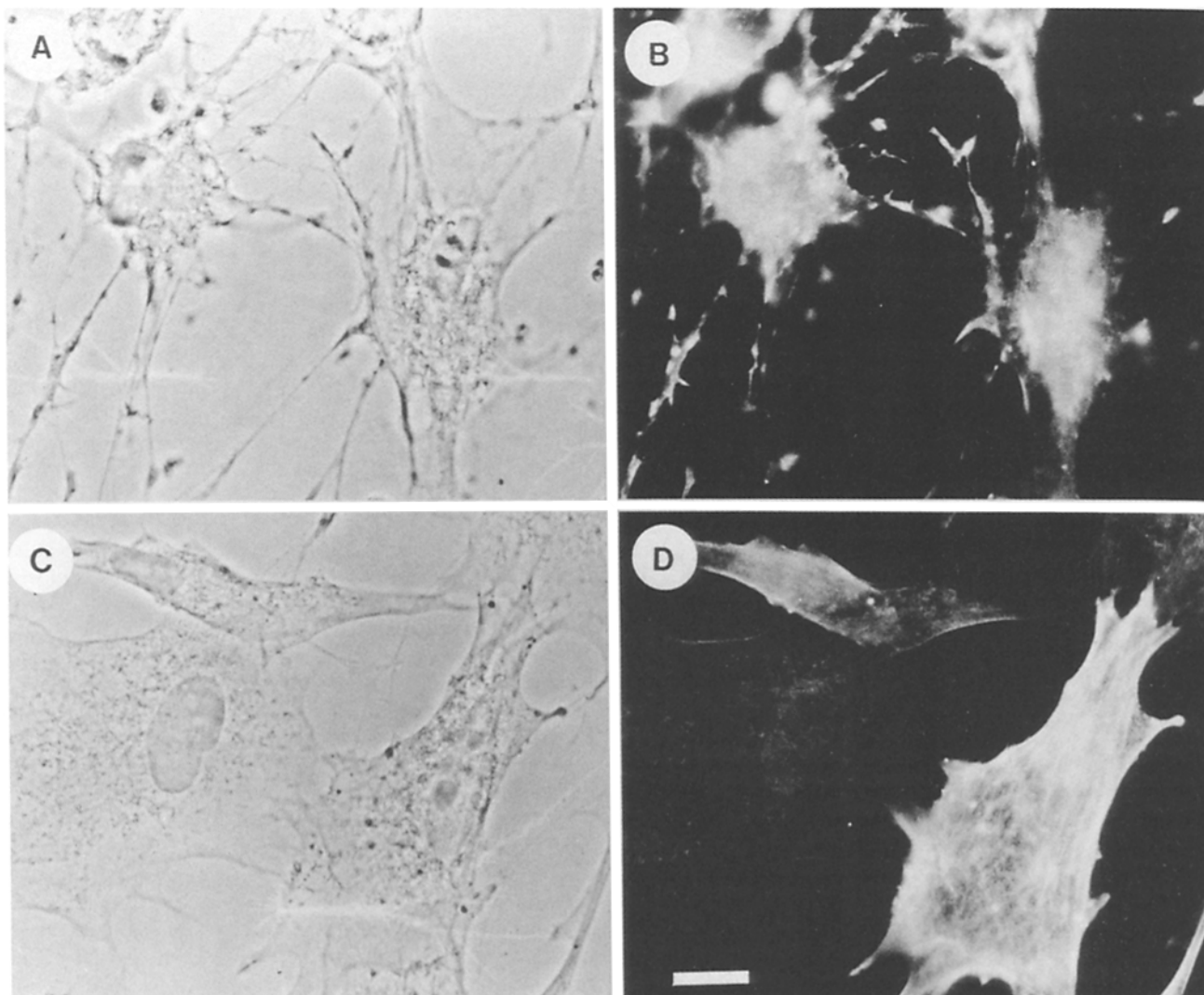
Figure 1. Indirect immunofluorescence of CEF cells at either 30 min after start of a cytochalasin B treatment (*A* and *B*), or 25 min after the reversal of cytochalasin B treatment (*C* and *D*). Cells were stained with monoclonal antibody CG1. (*A* and *C*) phase-contrast micrographs; (*B* and *D*) fluorescent micrographs. Bar, 10 μm.

all of the CEF cells showed no CG1 staining except for a faint nuclear fluorescence (data not shown). The possibility that Triton extraction may remove tropomyosin has also been examined by two-dimensional gel analysis. While 20–40% of tropomyosin was extracted with 0.5% Triton X-100 from CEF cells, no significant amounts of tropomyosin, including isoform 3, were extracted with 0.05% Triton X-100. Cells extracted by glycerol before Triton treatment and cells extracted with KTG solution (0.05% Triton X-100 and 30% glycerol in 0.1 M Pipes, pH 6.9, 5 mM $MgCl_2$, 0.2 mM EGTA) do not show the loss of CG1 staining exhibited by cells extracted in Triton alone. These results are not what would be expected if the CG1 antigenic determinant were masked by some other protein; detergent extraction of a protein obstructing the antibody-binding site should enhance staining, not block it. Also, the effect must be limited to the region of the CG1 determinant, since polyclonal antibodies, and even other monoclonal antibodies, show very bright stress fiber staining after Triton extraction (data not shown). For these reasons, the lack of CG1 staining after Triton ex-

traction argues against the possibility of a masking protein but indirectly supports a model in which the CG1 determinant exists in at least two conformational states, one of which is recognized by CG1 and one of which is not. This model provides a mechanism by which nonmuscle tropomyosin might play a regulatory role in the actin-myosin interaction. However, the direct evidence to support this model remains to be obtained.

To further investigate the CG1 antigenic determinant, we purified individual tropomyosin isoforms and carried out performic acid oxidation to force a change in conformation around the cysteine and methionine residues in the polypeptide. Then we used protein immunoblots to compare the reactivity of CG1 antibody with these proteins before and after oxidation. Fig. 4 shows the results of such blots. After oxidation, all three isoforms showed substantial changes in their mobilities in SDS–PAGE (Fig. 4 *A*). Before oxidation, tropomyosin isoform 1 was weakly recognized by the CG1 antibody. Surprisingly, its oxidized form showed a much stronger reactivity with the same antibody (Fig. 4 *B*). Con-
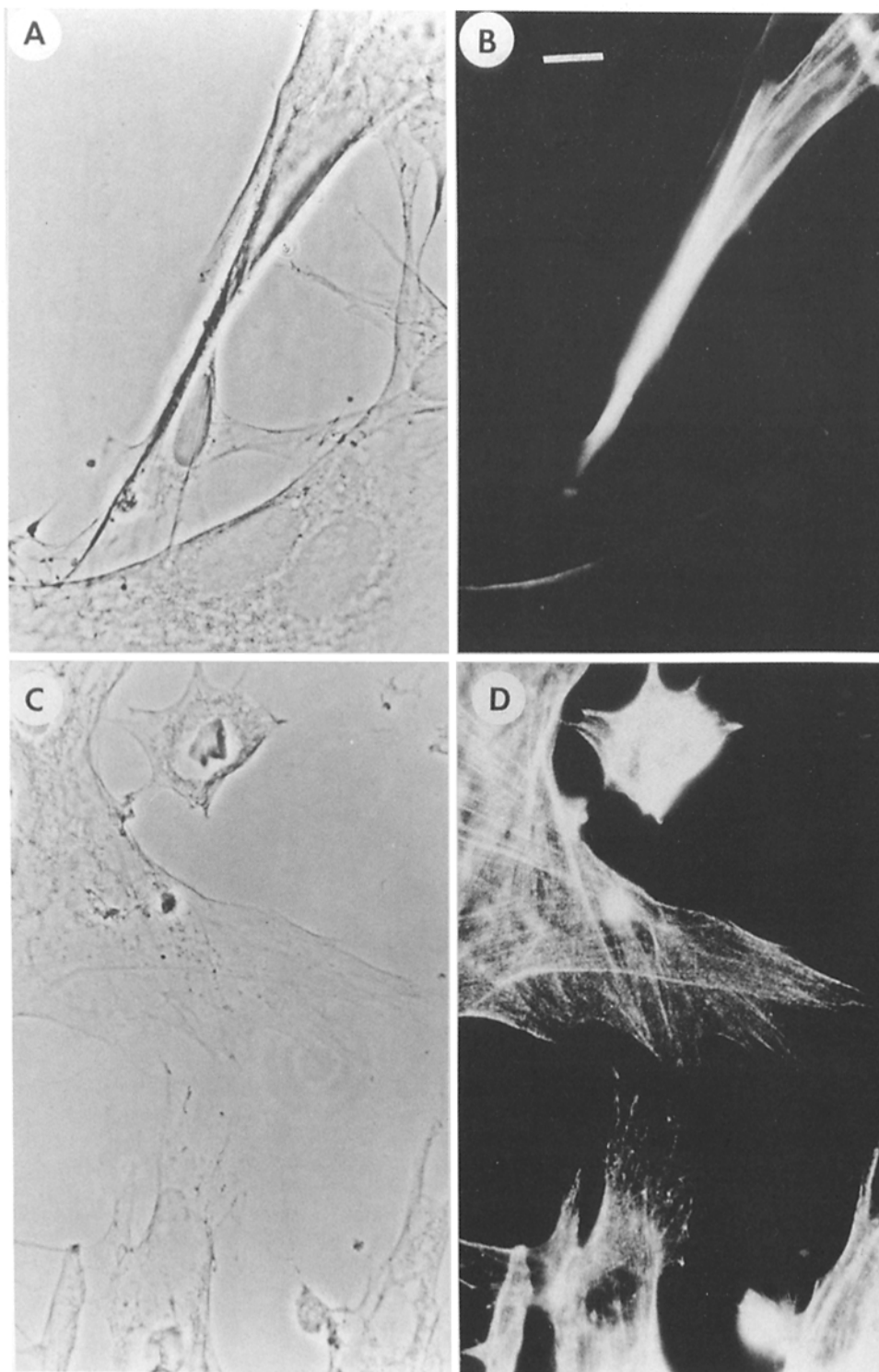
Figure 2. Indirect immunofluorescence of control (*A* and *B*) and glycerinated (*C* and *D*) CEF cells stained with monoclonal antibody CG1. (*A* and *C*) phase-contrast micrographs; (*B* and *D*) fluorescent micrographs. Bar, 10 μm.

sistent with our previous report (23), neither isoform 2 nor its oxidized form were recognized by the CG1 antibody (Fig. 4 *B*). By contrast, tropomyosin isoform 3 was recognized very strongly by the CG1 antibody before oxidation, but the performic acid oxidation destroyed its antigenicity (Fig. 4 *B*). These results suggest that the antigenic determinant recognized by the CG1 antibody may involve the cysteine or methionine residues.

DTNB is a chemical that forms interchain disulfide cross-links between adjacent cysteine molecules. It has been used by Lehrer and colleagues (19, 20) to demonstrate the existence of two conformational states of the region around the Cys-190 residue of skeletal muscle tropomyosin. In the present study, we used DTNB in an experiment similar to the performic acid oxidation described above, except that the change in cysteine residue induced by DTNB is much more
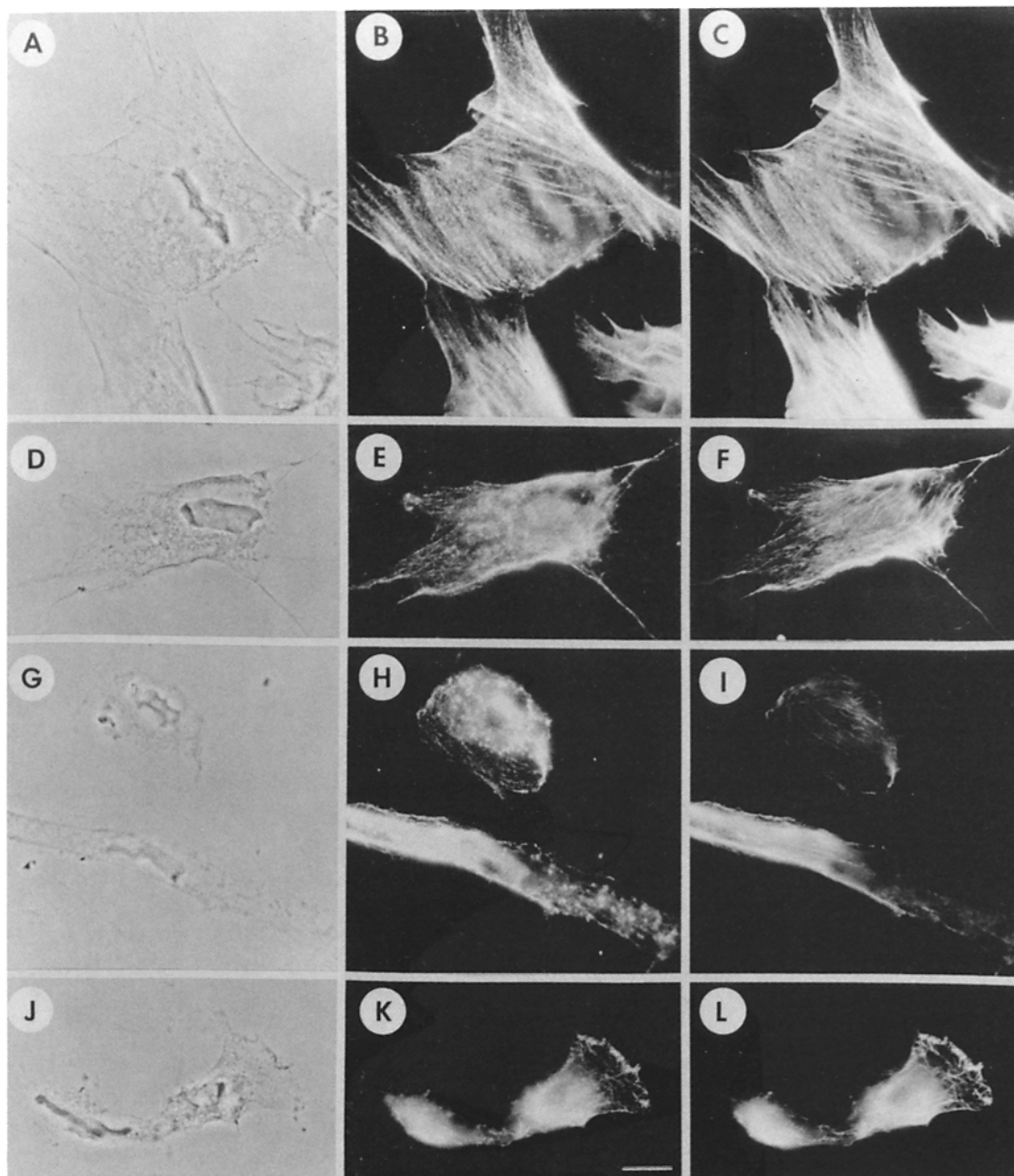
Figure 3. Indirect double-label immunofluorescence of contracted, glycerinated CEF cells stained with both monoclonal antibody CG1 and rabbit polyclonal antiserum against tropomyosin. (*A–L*) Glycerinated cells undergoing contraction at 0, 2, 10, and 20 min, respectively, after the addition of Mg-ATP. (*A, D, G,* and *J*) Phase-contrast micrographs; (*B, C, E, F, H, I, K,* and *L*) fluorescent micrographs. (*B, E, H,* and *K*) Cells reacted with CG1 antibody and detected with tetramethyl rhodamine isothiocyanate–conjugated goat anti-mouse IgG. Note that all the cells stain positively. (*C, F, I,* and *L*) Cells in the same fields seen in *B, E, H,* and *K*, respectively, reacted with rabbit anti-tropomyosin and detected with FITC-conjugated goat anti-rabbit IgG. Bar, 10 μm.

specific than that induced by performic acid oxidation. As can be seen from the blot stained with Amido black in Fig. 5, CEF tropomyosin after DTNB treatment forms a heterodimer of isoforms a and c, and homodimers of isoforms 1 and 3. The tropomyosin isoforms in this oxidized gel were identified by two-dimensional SDS–PAGE with the oxidized condition as the first dimension and the reduced condition as the second condition (25).

Immunoblot analysis of purified tropomyosin treated with DTNB showed that CG1 recognized monomers of isoforms 1 and 3, but did not recognize homodimers of dimers involving these isoforms (Fig. 5). Another monoclonal antibody, CGβ6, reacts with monomers of isoforms a, b, 1 and 2, as well as any dimers that contain one or more of these isoforms (a–c and 1–1). Thus, the change in cysteine residue induced by DTNB crosslinking prevented CG1 staining.
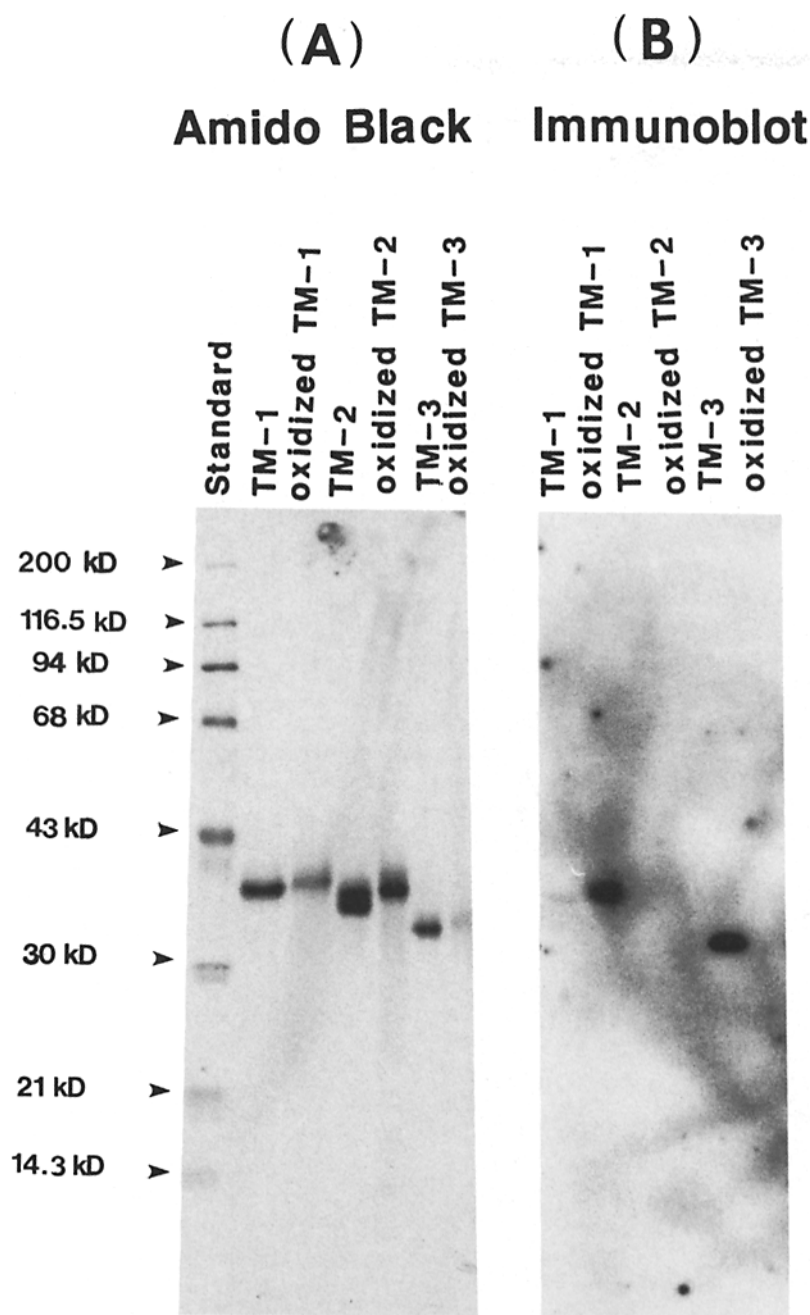
(A)
Amido Black

(B)
Immunoblot

Standard
TM-1
oxidized TM-1
TM-2
oxidized TM-2
TM-3
oxidized TM-3

TM-1
oxidized TM-1
TM-2
oxidized TM-2
TM-3
oxidized TM-3

200 kD ➤
116.5 kD ➤
94 kD ➤
68 kD ➤
43 kD ➤
30 kD ➤
21 kD ➤
14.3 kD ➤

Figure 4. Protein immunoblot analysis of monoclonal antibody CG1 binding to purified CEF tropomyosin isoforms and their respective oxidized products obtained by performic acid oxidation. Purified tropomyosin isoforms (*TM-1*, *TM-2*, and *TM-3*) and their oxidized products were separated on SDS–PAGE. After electrophoresis, proteins were transferred to nitrocellulose paper and either stained with Amido black (*A*) or immunoblotted with monoclonal antibody CG1 (*B*). The reactivity of CG1 antibody to *TM-1* greatly increases after oxidation of *TM-1*. On the other hand, the loss of reactivity is evident for the oxidized form of *TM-3*.

Discussion

The heterogeneity of the CG1 staining pattern does not appear to be artifactual. The pattern is not changed at different concentrations of antibody, nor is it the result of a mixture of cell types, since our CEF cell cultures show very little contamination by other cell types (26). Second antibodies labeled with tetramethyl rhodamine isothiocyanate or FITC both allow visualization of the heterogenous staining pattern.

Since all cells are strongly positive to CG1 staining after glycerination or cytochalasin B treatment, it is likely that the CG1 determinant (or tropomyosin isoforms 1 and 3) is present in all cells. From two-dimensional gel analysis of [35S]methionine-labeled single cells, we have noted that all tropomyosin isoforms could be detected in the five cells tested (data not shown). These results further support the idea that every cell contains the antigenic determinant recognized by CG1 antibody, but that this determinant is not accessible for antibody binding in some populations of cells.

Whether this heterogenous staining pattern reflects functional differences of tropomyosins or microfilaments is not known. Based on the results with cells partially synchronized with colcemid, this selective masking seems independent of cell cycle. There are many possible explanations for the unmasking effect of glycerol and cytochalasin B, such as the extraction of a masking protein or the induction of a conforma-
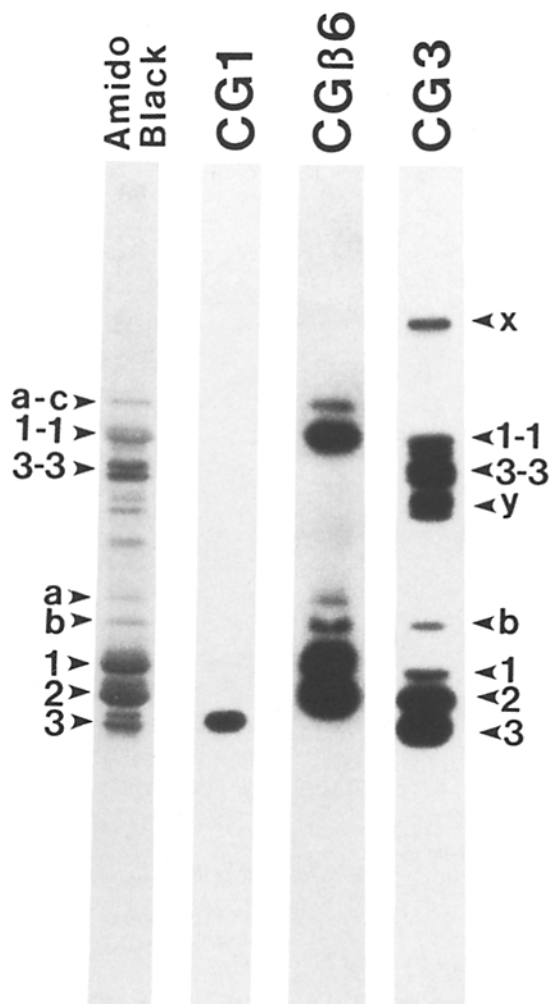
Figure 5. Protein immunoblot analysis of monoclonal antibodies CG1, CGβ6, and CG3 binding to CEF tropomyosin isoforms and the isoform dimers (1-1, 3-3, a–c) formed after DTNB treatment. CG1 recognizes *TM-1* and *TM-3* monomers. CGβ6 recognizes isoforms a, b, 1, and 2 as well as dimers involving any of these isoforms. Similarly, CG3 recognizes isoforms 1, 2, and 3 and also recognizes dimers involving any of these species. (*x* and *y*) Unknown proteins, detected by CG3 antibody. They may be the result of dimerization of any of the CEF tropomyosin isoforms with other contaminant proteins in our partially purified preparation. They failed to be identified in our Coomassie Blue-stained two-dimensional SDS-polyacrylamide gel (25).
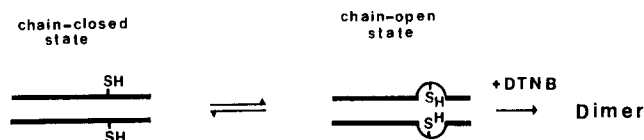

Figure 6. Schematic model for the two conformational states of skeletal tropomyosin proposed by Lehrer and colleagues (2, 20). A stable chain-closed state and a somewhat less stable chain-open state have been detected in the region near the Cys-190 residue (indicated by SH) of native skeletal tropomyosin. The existence of this chain-open state gives considerable flexibility to skeletal tropomyosin, thereby conferring its regulatory role in muscle contraction. This chain-open, locally unfolded state can also explain the ability of 5,5'-dithiobis (2-nitrobenzoate) to form a disulfide cross-link between the subunits of skeletal tropomyosin.

would not be expected to cause a decrease in staining intensity. This reasoning is supported by the observation that glycerol is able to stabilize the tropomyosin in some way which prevents the loss of CG1 staining after detergent treatment.

The skeletal tropomyosin on thin filaments is believed to undergo a conformational change in response to a change in Ca^{++} concentration. Through this change, tropomyosin is able to regulate interactions between actin and myosin, leading to muscle contraction and relaxation (8, 18). Two conformational states (a stable chain-closed state and a chain-open state) have been detected in the region near the Cys-190 residue of native skeletal tropomyosin in solution (2, 19, 20). A schematic representation of these states is shown in Fig. 6. The existence of this chain-open state gives considerable flexibility to skeletal tropomyosin, making possible its regulatory role in muscle contraction (2, 20). This chain-open, locally unfolded state can also explain the ability of 5,5'-dithiobis (2-nitrobenzoate), an interchain disulfide cross-linker, to form disulfide cross-links between subunits of both skeletal and cardiac tropomyosins by oxidation of opposing cysteine residues (19). We have previously shown that CEF tropomyosin isoforms 1 and 3, but not 2, are able to form cross-linked homodimers under the same conditions as striated tropomyosins form dimers, suggesting that these isoforms have a region analogous to the Cys-190 region of skeletal tropomyosin. Coincidently, the CG1 antibody recognizes isoforms 1 and 3, but not 2 (23). It is possible that the antigenic determinant recognized by CG1 antibody is the region near Cys-190, and that this localized region on isoforms 1 and 3 can exist in two different conformational states (chain-open and chain-closed). For this reason, we targeted cysteine residues, and the presumed Cys-190 residue in particular, in our attempts to force changes in the tropomyosin molecule with performic acid oxidation and DTNB treatment. The results we have presented indicate that the reactivities of the CG1 antibody to its antigens can be artificially altered to become either stronger or weaker after performic acid oxidation of tropomyosin isoform 1 or 3, respectively. This suggests that the CG1 antibody may recognize a determinant involving the cysteine residue. In addition, CG1 recognizes monomers of isoforms 1 and 3 but not the homodimers formed after DTNB treatment. This evidence, combined with the fact that the majority of the morphologically-identifiable motile cells stain strongly positive with this antibody, makes it reasonable to postulate that the determinant recog-

tional change in a masking protein or in the tropomyosin molecule itself. However, treatment with 2 M urea or mild trypsin, chymotrypsin, or V.8 protease, which in other cases has shown the ability to extract or loosen masking components (9), has failed to change the heterogenous staining pattern. Nonionic detergent extraction of CEF cells prevents staining by CG1 but does not prevent staining by other antibodies specific to tropomyosin. Furthermore, the detergent at 0.05% concentration does not extract the tropomyosin itself, including isoform 3, as judged by two-dimensional gel analysis. The lack of CG1 staining after detergent extraction is much more easily explained by a change of conformation in the CG1 antigenic determinant than by the existence of a masking protein, since the extraction of a masking protein

nized by CG1 may undergo a motility-dependent change within the living cells. Whether this change is due to conformational change remains to be determined.

Is the antigenic determinant recognized by CG1 antibody one of the important regions for tropomyosin function? To test this possibility, we have started experiments with microinjection of this antibody into living cells. Preliminary results show that cells injected with intact antibody change their morphology and collapse into tight balls. When Fab fragments of CG1 antibody are used, many of the injected cells retain their spread morphology but cease their intracellular granule movement (11). A detailed report will be published elsewhere.

We and other investigators have previously shown (a) that nonmuscle cells so far examined express multiple isoforms of tropomyosin; (b) that major tropomyosin isoforms are greatly reduced and minor isoforms are increased in many different types of transformed cells (12, 17, 21, 25, 27, 30); and (c) that minor isoforms with smaller apparent molecular mass appear to have a weaker actin-binding ability than major isoforms with higher apparent molecular mass (6, 7, 25, 31). In addition, we have used isoform-specific antibodies to demonstrate in some preliminary experiments that CEF tropomyosin isoform 3 has a different intracellular localization than other major isoforms (24). Together, these results suggest that the expression of various amounts of the different nonmuscle tropomyosin isoforms and the differential distribution of these isoforms in localized regions of the cell may be a critical component in the regulation of motility and shape maintenance by nonmuscle cells. The molecular mechanism by which nonmuscle tropomyosins regulate the actin-myosin interaction is still an open question. In the present study, a motility-dependent change in the availability of a determinant of nonmuscle tropomyosin has been detected by a monoclonal antibody. This, together with the recent reports (4, 29, 34) that both smooth muscle and nonmuscle cells contain a Ca^{++}/calmodulin-binding and actin-binding protein (caldesmon), may suggest a type of regulation analogous to the tropomyosin-troponin complex in striated muscles, although some differences between caldesmon and the troponin complex have been noted (4).

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