Differential Modulation of Actin-severing Activity of Gelsolin by Multiple Isoforms of Cultured Rat Cell Tropomyosin

POTENTIATION OF PROTECTIVE ABILITY OF TROPOMYSIN BY 83-kDa NONMUSCLE CALDESMON*

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Multiple isoforms of tropomyosin (TM) of rat cultured cells show differential effects on actin-severing activity of gelsolin. Flow birefringence measurements have revealed that tropomyosin isoforms with high M, values (high M, TMs) partially protect actin filaments from fragmentation by gelsolin, while tropomyosins with low M, values (low M, TMs) have no significant protection even when the actin filaments have been fully saturated with low M, TMs. We have also examined the effect of nonmuscle caldesmon on the severing activity of gelsolin because 83-kDa nonmuscle caldesmon stimulates actin binding of rat cell TMs (Yamashiro-Matsumura, S., and Matsumura, F. (1988) J. Cell Biol. 106, 1973–1983). While nonmuscle caldesmon alone or low M, TMs alone show no significant protection against fragmentation by gelsolin, the low M, TMs coupled with 83-kDa protein are able to protect actin filaments. Further, high M, TMs together with 83-kDa protein appear to block the severing activity completely. Electron microscopic analyses of length distribution of actin filaments have confirmed the results. The average length of control actin filaments is measured as 1.46 ± 1.0 μm, and gelsolin shortens the average length to 0.084 ± 0.039 μm. Similar short average lengths are obtained when gelsolin severs actin complexed with low M, TMs (0.080 ± 0.045 μm) or with nonmuscle caldesmon (0.11 ± 0.072 μm) while longer average length (0.22 ± 0.18 μm) is measured in the presence of high M, TMs. The simultaneous addition of nonmuscle caldesmon makes the average length considerably longer, i.e. 0.61 ± 0.37 μm in the presence of low M, TMs and 1.57 ± 0.87 μm in the presence of high M, TMs. Furthermore, the actin binding of gelsolin is strongly inhibited by co-addition of high M, TMs and nonmuscle caldesmon. These results suggest that TM and gelsolin share the same binding site on actin molecules and that the differences in the actin affinities between TMs are related to their abilities of protection against gelsolin.

A variety of actin-binding proteins interact, directly or indirectly, with each other and regulate the actin assembly dynamics (for review, see Refs. 1–3). We have been interested in the interrelationships between tropomyosin (TM) and actin-binding proteins because we (4, 5), as well as others (6–11), have suggested that TM is intimately related to the reorganization of microfilaments after various transformations of cultured cells. In cultured rat cells, as many as five different isoforms of tropomyosin have been identified (12). Of these isoforms, TM isoforms with higher M, values (high M, TMs) are predominant in normal cells. They are replaced with TM isoforms with lower M, values (low M, TMs) in morphological alterations associated with cell transformation (4). Biochemical characterization has revealed that high M, TMs (prominent in normal cells) show a higher affinity for actin and a greater ability to polymerize in a head-to-tail fashion than do low M, TMs (prominent in transformed cells) (13). Furthermore, we have demonstrated that an actin-binding protein with M, of 55,000 isolated from HeLa cells (55-kDa protein) differentially modulates the actin binding of the multiple TM isoforms of cultured cells, depending on the TM isoform types (14, 15). 55-kDa protein inhibits the binding of low M, TMs but not high M, TMs to actin. These results suggest that the multiple TM isoforms play different functions in the organization of microfilaments in transformed cells through the modulation of interactions between actin and actin-binding proteins.

Other laboratories have also shown that TM alters the activities of a variety of actin-binding proteins. It has been reported that skeletal muscle TM inhibits binding of filamin, spectrin, and α-actinin to actin (16–19). TM also inhibits the actions of fragmin, villin, gelsolin, DNase I, and actin-depolymerizing factor on actin (20–25). It seems, however, necessary to examine whether the multiple TM isoforms of cultured cells, differentially modulate the interaction between actin and actin-binding proteins of cultured cells, because the multiple TM isoforms differ from each other and from muscle TM in their actin-binding abilities (13). In fact, cultured cell TMs (either high or low M, TMs) do not inhibit the binding of filamin to actin, unlike skeletal muscle TM.

Gelsolin is an actin-binding protein that is widely distributed in a variety of animal cells including cultured cells (26, 27; also for review, see Refs. 1–3). The protein shows three functions, i.e. severing actin filaments into short ones, promoting nucleation for actin polymerization, and capping barbed ends of actin filaments. Its actin-severing activity is

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1 The abbreviations used are: TM, tropomyosin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride.

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regulated in a calcium-dependent way. Further, Stossel and co-workers (22) have reported that skeletal muscle TM and macrophage nonmuscle TM inhibits the severing activity of gelsolin. Because most transformed cells show changes in the assembly of microfilaments from bundles to a dispersed state (25), gelsolin and TM are candidates that may play a role in such reorganization. We are, therefore, interested in determining if the multiple TM isoforms differentially modulate the interactions between actin and gelsolin. As 83-kDa nonmuscle caldesmon stimulates actin binding of cultured cell TMs (29), its effects on gelsolin severing activity was also examined.

In this report, we describe that high $M$, TMs but not low $M$, TMs can effectively inhibit gelsolin from producing short filaments. While 83-kDa protein alone does not have any protective effect against gelsolin, the addition of 83-kDa nonmuscle caldesmon greatly potentiates the protective ability of TMs against gelsolin. We discuss our results in terms of microfilament organization in normal and transformed cells.

MATERIALS AND METHODS

Proteins—A mixture of five TM isoforms were prepared from cultured rat cells, REF-WT4A cells, and were separated into high $M$, (a mixture of TM-1 and TM-2) and low $M$, (a mixture of TM-4 and TM-5) TMs by chromatography on hydroxyapatite as described previously (13). Muscle actin and TM were purified from rabbit skeletal muscle as described (4). Nonmuscle caldesmon with a $M$, of 83,000 were purified from REF-WT4A cells as described previously (30).

Rabbit plasma gelsolin was purified by the methods of Harris and Schwartz (31) and Harris and Weeds (32) with slight modification. Briefly, the 40-60% ammonium sulfate fraction of 50 ml of rabbit serum was dialyzed overnight against 20 mM Tris-HCl (pH 8.0) and loaded on a CM-cellulose column (0.8 X 90 cm) equilibrated with the same Tris buffer. Proteins were eluted by a linear NaCl gradient (0-0.4 M) in the same buffer. Active fractions were collected, dialyzed against 5 mM sodium phosphate buffer (pH 6.8), 0.6 M NaCl, and 0.2 mM PMSF.

Gelsolin fractions, eluted at 30 mM phosphate, were dialyzed against 5 mM succinate buffer (pH 6.0), 0.2 mM PMSF, and 0.01 mM EDTA and loaded on a CM-cellulose column (0.8 X 30 cm) equilibrated with the same succinate buffer. Active fractions were eluted at 100 mM NaCl were pooled and concentrated using a Centricon devise with YM-30 membranes (Amicon). Final purification was achieved by gel filtration on a column (1.2 X 90 cm) of Sephacryl S-200 (Pharmacia LKB Biotechnology, Inc.) equilibrated with 50 mM Tris-acetate buffer (pH 7.6), 0.1 M NaCl, and 0.2 mM PMSF. Approximately 0.2 mg of gelsolin with a purity of more than 96% was obtained.

Assays for Actin-severing Activity of Gelsolin—The severing activity of gelsolin was measured by the following two methods. 1) Flow birefringence. Flow birefringences of F-actin solutions (final concentration of 12 $\mu$M) were determined with a Micro FBR MARK II (Wakenyaku Co., Japan) at the rotation speed of 500 rpm. The apparatus consists of a polarizing microscope with a rotatable specimen stage, thus allowing us to measure flow birefringences as little as 150 $\mu$l of solution. 2) Electron microscopy. Length distributions of F-actin in the presence or absence of gelsolin or TMs at 83-kDa nonmuscle caldesmon were examined with an electron microscope using the negative staining technique. Length distributions of F-actin in the presence or absence of gelsolin or TMs at 83-kDa nonmuscle caldesmon were examined with an electron microscope using the negative staining technique. Length distributions of F-actin in the presence or absence of gelsolin or TMs at 83-kDa nonmuscle caldesmon were examined with an electron microscope using the negative staining technique. Length distributions of F-actin in the presence or absence of gelsolin or TMs at 83-kDa nonmuscle caldesmon were examined with an electron microscope using the negative staining technique. Length distributions of F-actin in the presence or absence of gelsolin or TMs at 83-kDa nonmuscle caldesmon were examined with an electron microscope using the negative staining technique. Length distributions of F-actin in the presence or absence of gelsolin or TMs at 83-kDa nonmuscle caldesmon were examined with an electron microscope using the negative staining technique. Length distributions of F-actin in the presence or absence of gelsolin or TMs at 83-kDa nonmuscle caldesmon were examined with an electron microscope using the negative staining technique. Length distributions of F-actin in the presence or absence of gelsolin or TMs at 83-kDa nonmuscle caldesmon were examined with an electron microscope using the negative staining technique. Length distributions of F-actin in the presence or absence of gelsolin or TMs at 83-kDa nonmuscle caldesmon were examined with an electron microscope using the negative staining technique.

Effects of TMs on the severing activity of gelsolin were measured using the following two sets of conditions. 1) F-actin (final concentration, 12 $\mu$M) was first incubated for 1 h at room temperature with constant concentrations of skeletal muscle TM (2.1 $\mu$M), high $M$, TMs (2.1 $\mu$M), or low $M$, TMs (2.4 or 5.3 $\mu$M) in 100 mM KCl, 0.1 mM CaCl$_2$, 1 mM MgCl$_2$, 1 mM ATP, 20 mM imidazole buffer (pH 7.0), and then varying concentrations (0-0.27 $\mu$M) of gelsolin were added, to make a total volume of 150 $\mu$l. After incubation for 40 min, flow birefringences and length distributions of F-actin were measured as described above. 2) F-actin (final concentration, 12 $\mu$M) was first incubated for 1 h at room temperature with constant concentrations of 83-kDa nonmuscle caldesmon alone (2.4 $\mu$M), or TMs alone (2.1 $\mu$M for high $M$, TMs or 5.2 $\mu$M for low $M$, TMs) or both 83-kDa nonmuscle caldesmon and TMs. Then varying amounts of gelsolin (0-0.27 $\mu$M) were added. The salt conditions are the same as described above. After 40 min, flow birefringence and length distributions of F-actin were measured. 2) F-actin (final concentration, 12 $\mu$M) was first incubated for 1 h at room temperature with varying concentrations (0-4.8 $\mu$M) of 83-kDa protein with or without 5.2 $\mu$M low $M$, TMs, and then 0.27 $\mu$M gelsolin was added. After 40 min, severing activities were measured by flow birefringence.

Actin Binding of Gelsolin—F-actin (final 24 $\mu$M) was incubated for 1 h with buffer, or high $M$, TMs (6.1 $\mu$M) or 83-kDa nonmuscle caldesmon (3.0 $\mu$M), or both, and then 0.27 $\mu$M gelsolin was added. After 40 min, samples of 60 $\mu$l were centrifuged at 30 p.s.i. for 30 min with a Beckman Airfuge. The amounts of actin and gelsolin in both pellets and supernatants were determined quantitatively by densitometry as described (14, 15, 29). Briefly, both pellets and supernatants were dissolved in equal volumes of SDS sample buffer. Samples were run on 12.5% SDS-polyacrylamide gel, stained with Coomassie Brilliant Blue R-250, and scanned with a densitometer (Joyce Loehl Chromoscan 3, Vickers Instrument Inc., Malden, MA). The linear range of dye binding was determined for each protein, and quantitation was carried out within the limits of linear ranges.

Other Procedures—Protein concentrations were determined by the method of Lowry et al. (33) using bovine serum albumin as a standard. SDS-PAGE was performed essentially as described by Blattler et al. (34) using 12.5% polyacrylamide except that the buffer system of

![Fig. 1. Effects of gelsolin on the flow birefringence of actin fila-
ments in the presence and absence of different TM iso-
forms.](image-url)
Laemmli (35) was used. Protein samples were solubilized in an equal volume of 2 x SDS sample buffer at a final concentration of 1% SDS, 50 mM dithiothreitol, 40 mM Tris-HCl, pH 6.8, 7.5% glycerol, and 0.00025% bromphenol blue.

RESULTS

Effects of TM Isoforms on F-actin-severing Activity of Plasma Gelsolin—We first examined whether high M, or low M, TMs have different effects on the F-actin-severing activity of plasma gelsolin. F-actin was complexed with fixed concentrations of either high or low M, TMs from cultured cells, and then varying amounts of gelsolin were added.

As Fig. 1 shows, high M, TMs, but not low M, TMs, protect F-actin from fragmentation by gelsolin. In the absence of TMs, the flow birefringence of F-actin solution (12 μM) quickly dropped from 20° to 5° within 2 min of gelsolin (0.27 μM) addition, suggesting that gelsolin severs F-actin filaments into short ones. A similar quick drop in birefringence was also observed in the presence of either 2.4 or 5.2 μM low M, TMs. On the contrary, F-actin complexed with 2.1 μM high M, TMs showed a relatively small decrease (from 27° to 21°) in the birefringence even after 1 h. A similar protection was also observed when F-actin was complexed with 2.1 μM skeletal muscle TMs.

We have then examined effects of TM concentrations on the inhibition of gelsolin severing activity (Fig. 2). F-actin (12 μM) was first incubated with varying concentrations of either high (0-4.5 μM) or low (0-17.2 μM) M, TMs and gelsolin (0.27 μM) was added. Actin binding of the TMs in the absence of gelsolin was measured at the same time to confirm the saturation.

As Fig. 2A shows, high M, TMs are able to protect actin filaments against gelsolin. The birefringence of F-actin was increased from 5° to 22° as high M, TMs saturated F-actin.
Activity concentration protective effects, simultaneous addition of both proteins protects birefringence of actin in the presence of both low concentrations (0-4.8 gelsolin was determined by densitometry and blotted by the dotted line (X)). While 83-kDa protein alone or low M, TMs alone has no protective effects, simultaneous addition of both proteins protects actin filaments from severing.

The increase in the flow birefringence is roughly proportional to the extent of actin binding of the TM.

On the contrary, low M, TMs have little protective ability (Fig. 2B), confirming the results of Fig. 1. Because low M, TMs have a lower affinity (K_{app} = 4.4 \times 10^8 M^{-1}, Ref. 29) for actin than do high M, TMs (K_{app} = 6.7 \times 10^8 M^{-1}, Ref. 29), we increased the concentration of low M, TMs up to 17.2 \mu M, the concentration which was twice higher than that needed for the full saturation (see actin binding of Fig. 2B). Even at this high concentration, the birefringence of F-actin was just slightly increased from 5° to 8°.

In the absence of gelsolin, the flow birefringence was increased from 20° to 27° as either high or low M, TMs saturated F-actin, probably because the binding of TM inhibited spontaneous fragmentation caused by rotational flow.

Effects of 83-kDa Nonmuscle Caldesmon on the Severing Activity of Gelsolin—We have examined whether 83-kDa nonmuscle caldesmon inhibits severing activity of gelsolin, because we have suggested that 83-kDa nonmuscle caldesmon binds to actin along the length of filaments in a way that resembles TM binding (29, 30). Further, like TM, 83-kDa nonmuscle caldesmon from cultured rat cells is a rod-like, heat-stable protein, and binds to actin at a saturation ratio of one 83-kDa protein to six actin monomers.

Unlike high M, TMs or skeletal muscle TMs, 83-kDa nonmuscle caldesmon does not appear to inhibit the actin-severing activity of gelsolin. In Fig. 3A, actin (12 \mu M) was first complexed with 83-kDa protein alone or low M, TMs, and then gelsolin was added. Actin binding of 83-kDa protein in the absence of gelsolin was determined by densitometry and blotted by the dotted line (X). While 83-kDa protein alone or low M, TMs alone has no protective effects, simultaneous addition of both proteins protects actin filaments from severing.

We have previously shown that 83-kDa protein greatly stimulates actin binding of low M, TMs and increases the apparent association constants from 4.4 \times 10^8 to 1.5 \times 10^8 M^{-1} (29). We have thus examined whether such an increase changes the properties of low M, TMs in such a way that low M, TMs protect against gelsolin. As Fig. 3B shows, while 83-kDa protein alone or low M, TMs alone do not inhibit actin-severing activity, 83-kDa protein together with low M, TMs is able to block severing activity as effectively as high M, TMs (see Fig. 3C).

We have also examined if 83-kDa nonmuscle caldesmon has any effect on the protective ability of high M, TMs against gelsolin. As Fig. 3C shows, high M, TMs coupled with 83-kDa protein block the severing activity of gelsolin almost completely. The flow birefringence in this case was not decreased significantly by increasing the concentration of gelsolin up to 0.27 \mu M.

Because the amount of nonmuscle caldesmon used in the above experiments is not saturating, we have asked if a saturating amount of 83-kDa protein can inhibit gelsolin severing activity. Actin was complexed with increasing amounts (0-4.8 \mu M) of nonmuscle caldesmon and then 0.27 \mu M gelsolin added. Actin binding of 83-kDa nonmuscle caldesmon in the absence of gelsolin was measured at the same time to confirm the saturation. As Fig. 4 shows, actin fila-
Effects on Actin-severing Activity of Gelsolin by Rat Cell TM

FIG. 6. Length distribution of actin filaments in the presence and absence of gelsolin, TMs, and 83-kDa protein. Lengths of 300 actin filaments were measured from the electron micrographs taken in the same conditions as described in the legend of Fig. 5. Panels A–G correspond to those of Fig. 5. Mean length: A, 1.46 ± 1.0 μm; B, 0.964 ± 0.039 μm; C, 0.880 ± 0.045 μm; D, 0.61 ± 0.37 μm; E, 0.22 ± 0.18 μm; F, 1.57 ± 0.97 μm; G, 0.11 ± 0.072 μm.
were overloaded on SDS gels to show gelsolin bands clearly. 83-kDa protein and high a Beckman Airfuge. The pellets containing equal amounts of actin plexed with 83-kDa protein; added. After molecular weight markers; TMs and 83-kDa protein. The flow birefringence increased from 6° to 19° as gelsolin. The flow birefringence of F-actin. As reported previously (29), 2 kDa Nonmuscle Caldesmon on Gelsolin Severing Activity—ments saturated with 83-kDa protein were as sensitive to gelsolin as control actin filaments. On the other hand, co-addition of 5.2 μM low M, TMs made F-actin resistant to gelsolin. The flow birefringence increased from 6° to 19° as the concentrations of 83-kDa protein were increased to 2 μM and further addition of 83-kDa protein did not increase the flow birefringence of F-actin. As reported previously (29), 2 μM 83-kDa protein is high enough to stimulate the binding of gelsolin, suggesting that the high M, TMs partially inhibited the severing activity of gelsolin.

Effects on Actin-severing Activity of Gelsolin by Rat Cell TM

Electron Microscopic Analyses of the Effects of TMs and 83-kDa Nonmuscle Caldesmon on Gelsolin Severing Activity—The effects of TMs on the severing activity of gelsolin were also examined by electron microscopy. As Fig. 5 shows, long F-actin filaments of the control (A) were severed into very short ones by addition of gelsolin (B). Gelsolin also fragments F-actin when actin was complexed with either low M, TMs alone (C) or with 83-kDa protein alone (G). On the other hand, longer F-actin filaments were observed when F-actin was complexed with high M, TMs (E).

The addition of 83-kDa protein to TM-complexed actin has revealed remarkable effects on the lengths of F-actin filaments. Actin filaments complexed with both low M, TMs and 83-kDa protein (D) were found to be considerably longer than those complexed with low M, TMs alone or with 83-kDa protein alone. In the presence of high M, TMs and 83-kDa protein (F), the filaments became even longer and appeared to be as long as the control F-actin without gelsolin (A). It is also noticeable that those filaments appeared straighter than the control filaments, which is characteristic to TM-containing actin filaments.

To confirm these results, we have measured the length distribution of about 300 F-actin filaments in each case (Fig. 6). The control actin filaments without gelsolin showed a rather wide distribution of lengths (roughly distributed from 0.07 to 4.9 μm), and the average length was measured as 1.46 ± 1.0 μm (A). The addition of gelsolin made the average length about 18 times shorter, i.e. 0.084 ± 0.039 μm (B). Gelsolin also severed actin filaments complexed with either low M, TMs (C) or 83-kDa protein (G) into short ones with similar average lengths (0.086 ± 0.045 μm for actin-low M, TM complex and 0.11 ± 0.072 μm for actin-83-kDa protein complex) although actin complexed with 83-kDa protein appeared slightly longer than that with low M, TMs.

The presumption of actin filaments with high M, TMs (E), on the other hand, made the average length roughly three times longer, i.e. 0.22 ± 0.18 μm, than those of actin filaments in the absence of TMs or in the presence of either low M, TMs or 83-kDa protein. However, this length was still seven times shorter than that of control actin filaments without gelsolin, suggesting that the high M, TMs partially inhibited the severing activity of gelsolin.

The length measurement has also confirmed the effect of co-addition with 83-kDa protein. The average length of actin filaments complexed with both 83-kDa protein and low M, TMs was measured as 0.61 ± 0.37 μm. This value was considerably longer than that (0.22 ± 0.18 μm) of actin filaments in the presence of high M, TMs only, although the flow birefringence values were similar in both cases (see Fig. 3, B and C). While the reason for this discrepancy is currently unknown, this may suggest that flow birefringence is not exactly proportional to the length of actin filaments. For example, actin filaments over certain length may align in a rotational field to a similar extent as longer ones do, thus yielding similar value of flow birefringence.

Actin filaments complexed with both 83-kDa protein and high M, TMs showed a very similar length distribution with a similar average length of 1.57 ± 0.97 μm, indicating that high M, TMs together with 83-kDa protein almost completely blocked the severing activity of gelsolin.

Actin Binding of Gelsolin Is Inhibited by TM Complexed with 83-kDa Protein—It is important to determine whether TM inhibit severing activity of gelsolin or inhibit separation of the severed fragments. Hence, we have asked whether TM and nonmuscle caldesmon inhibit the binding of gelsolin to actin. At 0.27 μM gelsolin and 12 μM actin, however, only 30% of total actin was precipitated by high speed centrifugation, making the detection of the actin binding of gelsolin quite difficult. We have therefore doubled the concentration of actin to 24 μM with the same concentration (0.27 μM) of gelsolin to make longer, pelletable F-actin. In this condition, the fraction of pelletable actin was increased to 65%, and the amounts of gelsolin in actin pellets became detectable by SDS-PAGE (Fig. 7). While the amount of pelletable actin was not significantly changed in the presence of 83-kDa nonmuscle caldesmon, the amounts were increased to 80% in the presence of high M, TMs, and to more than 90% in the presence of both high M, TMs and nonmuscle caldesmon. The levels of gelsolin bound to actin in these conditions were compared by loading the same amount of actin on SDS gels.

High M, TMs and high M, TMs together with nonmuscle caldesmon were found to inhibit the binding of gelsolin to actin. As Fig. 7 shows, the level of gelsolin binding was decreased to one-third of the control (lane 2) by high M, TMs (lane 4), while it appeared unchanged by nonmuscle caldesmon (lane 3). The simultaneous addition of high M, TMs and nonmuscle caldesmon further decreased the level of gelsolin binding to one-fifth (lane 5). These results indicate that high
M, TMs block severing activity of gelsolin by the inhibition of actin binding of gelsolin.

It is worthwhile to note that the binding of high M, TMs to actin appears not inhibited by gelsolin. Sedimentation assay followed by gel densitometry has revealed that there is no significant difference in the binding of high M, TMs either in the presence or in the absence of gelsolin (data not shown).

**DISCUSSION**

Possible Mechanisms for the Inhibition of Gelsolin Severing Activity by TM and Caldesmon—The protection by TMs against gelsolin severing activity is simply explained by assuming that TMs and gelsolin compete in actin binding, while sharing the same actin binding site on an actin molecule. This hypothesis is supported by the following observations. First, high M, TMs with higher actin affinity can effectively inhibit both actin binding and severing activity of gelsolin (Figs. 1, 2, and 7). Second, the enhancement of actin binding of TMs by nonmuscle caldesmon makes both low and high M, TMs more protective (Figs. 3 and 4). Finally, an increase in gelsolin concentration overcomes the inhibitory effect by high M, TMs. Flow birefringence of F-actin-high M, TM complex was observed to drop from 20° to 10° when the concentration of gelsolin was increased five times from 0.27 to 1.35 μM (data not shown).

If this hypothesis is the case, an increase in the concentration of low M, TMs should make low M, TMs protective against gelsolin. The increase up to 17.2 μM, however, failed to show any significant protection (Fig. 2B). One possibility is that this concentration may not be high enough because the apparent binding constant of low M, TMs is 15 times lower than that of high M, TMs. Alternatively, low M, TMs may not share an actin binding site with gelsolin, thus making low M, TMs unable to protect against gelsolin. It is possible that 83-kDa protein not only increased actin binding of low M, TMs but also changes the actin binding site of low M, TMs to the same site as that of gelsolin.

It should be worthwhile to note that TMs from intestinal epithelial cells and erythrocytes have been reported to show high affinity for actin (36, 37), although their molecular weight values are similar to that of the low M, TMs from cultured rat cells. This type of low M, TMs may exert different effects on the severing activity of gelsolin. Brush-border TM, in fact, has been reported to inhibit villin’s actin-binding activity (25).

83-kDa nonmuscle caldesmon itself neither has significant protection against gelsolin severing activity nor inhibits the actin binding of gelsolin (Figs. 3, 4, and 7). These results suggest that 83-kDa protein and gelsolin do not share the actin binding site. Alternatively, the longitudinal binding of 83-kDa nonmuscle caldesmon along actin filaments may have gaps where gelsolin could attack to sever actin filaments.

Possible Functions of Gelsolin, TM, and Nonmuscle Caldesmon in the Organization of Microfilaments—While intracellular calcium and phosphatidylinositol 4,5-biphosphate are shown to regulate gelsolin activity (26, 27, 38), our results suggest that TMs and caldesmon play an additional role in the regulation of severing activity of gelsolin. Intracellular localization of these three proteins in tissue-cultured cells may support this notion. TM and nonmuscle caldesmon are co-localized in stress fibers of tissue-cultured cells (29, 30, 39). Furthermore, Cooper et al. (40) have recently demonstrated that gelsolin has a diffuse distribution in living cells. It is thus possible that TM and caldesmon may inhibit the binding of gelsolin in these cells even in the presence of calcium. If this is the case, the occurrence of gelsolin severing activity (if it occurred in vivo) may require the dissociation of one of these two proteins from microfilaments, in addition to an increase in intracellular calcium concentrations. Further studies including quantification of TM, caldesmon, and gelsolin in living cells are needed to elucidate whether these proteins interact in this way to regulate the organization of microfilaments.

There are conflicting reports on experiments involving the microinjection of gelsolin into living cultured cells. Cooper et al. (41) have shown that microinjection of gelsolin has no effect on cell morphology or microfilament organization. On the contrary, Sanger et al. (42) have reported that the effects of the injection depend on cell type. While myofibrils in embryonic myotubes are resistant to gelsolin injected, stress fibers of fibroblasts or epithelial cells are broken down. Although this discrepancy appears difficult to explain, it is possible that TM and caldesmon may be involved in the regulation of activity of gelsolin. For example, some cells may contain more TM isoforms with high actin affinity and/or higher levels of nonmuscle caldesmon, so that these cells may be resistant to gelsolin injected.

Although the in vivo functions of gelsolin, as well as those of TMs are yet to be elucidated, it is tempting to speculate how both TM and gelsolin are involved in the reorganization of microfilaments upon cell transformation. We have previously shown that low M, TMs with lower affinity to actin are predominant in most transformed cultured rat cells (4). In addition, levels of nonmuscle caldesmon were reported to be decreased in transformed cells (43). Furthermore, levels of 55-kDa actin binding protein that inhibits the binding of low M, TMs to actin (15), were increased in contrast to those of nonmuscle caldesmon. Thus all these changes are likely to make gelsolin more active and may cause fragmentation of actin filaments by gelsolin in cells. These changes may result in the morphological alterations observed in many transformed cells.

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