Purification and Characterization of Multiple Isoforms of Tropomyosin from Rat Cultured Cells*

(Received for publication, April 25, 1985)

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We have previously shown that rat cultured cells contain five isoforms of tropomyosin (Matsumura, F., Yamashiro-Matsumura, S., and Lin, J. J.-C. (1983) J. Biol. Chem. 258, 6636-6644) and that these tropomyosins are differentially expressed upon cell transformation (Matsumura, F., Lin, J. J.-C., Yamashiro-Matsumura, S., Thomas, G. P., and Topp, W. C. (1983) J. Biol. Chem. 258, 13954-13964). To examine functions of tropomyosin in microfilament organization, we have purified and partially separated the multiple isoforms of tropomyosin by chromatography on hydroxylapatite. Analyses of cross-linked dimers produced by air oxidation have revealed that all isoforms except the tropomyosin isoform with apparent Mr of 35,000 form homodimers. Although these tropomyosins share many properties characteristic of tropomyosin, structural analyses at a peptide level and immunological analyses have shown that the five isoforms can be classified into two groups, i.e., tropomyosins with higher apparent Mr (Mr = 40,000, 35,500, and 35,000) and tropomyosins with lower apparent Mr (Mr = 32,400 and 32,000). The low Mr tropomyosins show less ability for head-to-tail polymerization and lower affinity to actin than the high Mr tropomyosins. We suggest that these differences in properties may be related to the changes in microfilament organization observed in transformed cells.

Studies on skeletal muscle tropomyosin have shown that the protein is rod-shaped, composed of two highly α-helical subunits, and distributed along the axis of actin filaments to form continuous strands lying in both grooves of actin filaments (see Ref. 1 for review). The biological function of TM is well known in skeletal muscle. In association with the tropinin complex, TM regulates actomyosin interaction in a Ca2+-dependent manner (2, 3). TM has also been found in a variety of nonmuscle tissues and cultured cells including platelets (4-6), brain (7), sea urchin eggs (8), calf pancreas (9), cultured cells (9-15), rabbit lung macrophages (16), human erythrocyte (17), and bovine thyroid (18). The biochemical properties of nonmuscle TMs such as amino acid composition, isoelectric points, and α-helix contents are very similar to those of muscle TM (see Refs. 1 and 47 for review). The functions of TM in nonmuscle cells, however, are not clearly understood. Because tropinin has not been found in nonmuscle cells, the regulation of actomyosin by a tropinin-tropomyosin complex is not likely to occur in nonmuscle cells.

In the previous papers (11, 19), we have shown that rat cultured cells contain multiple isoforms of TM, and these TM isoforms are differentially expressed upon cell transformation. Using our method for the isolation of TM-containing microfilaments (11), we have found (i) in "normal" rat cultured cell lines, five proteins are identified as isoforms of TM, and of these, three are major TMs (TM-1 (Mr = 40,000), TM-2 (Mr = 36,500), and TM-4 (Mr = 32,400) and two are relatively minor TMs (TM-3 (Mr = 35,000) and TM-5 (Mr = 32,000)); (ii) upon transformation with either DNA or RNA viruses, the levels of one or both of the major TMs are decreased or missing and the levels of minor TMs are increased; (iii) the degree of these changes in TM patterns correlates well with the extent of morphological changes observed in transformed cells; and (iv) the changes in TM patterns are regulated at the level of mRNA. These results imply that alterations in the pattern of TM expression are involved in, or cause (in part), rearrangement of stress fibers. To permit the study of this possibility, we have purified and characterized these multiple isoforms of TM from rat cultured cells by biochemical, biophysical, and immunological methods. These analyses have revealed that the five isoforms of TM can be classified into two groups; one includes TM-1, TM-2, and TM-3 with higher Mr values on SDS gels, and the other includes TM-4 and TM-5 with lower Mr values. The group of TM-1, TM-2, and TM-3 shows higher ability for head-to-tail polymerization and higher affinity to actin than the other TM group (TM-4 and TM-5).

MATERIALS AND METHODS

Cell Culture—Cultured cells used in the present study were REF-52 cells (an established rat embryo cell line), an SV40 transformant (REF-WT4A) of REF-52, normal rat kidney cells (NRK, ATCC, CRL-1570), and Kirsten virus transformed NRK cells (ATCC, CRL-1569). All cell lines were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, and 5% CO₂ and 95% air at 37°C. For culture of REF-WT4A cells in a large scale, cells were grown in large plates (245 x 245 x 20 mm, Nunc) in the Dulbecco's modified Eagle's medium containing 10% calf serum. Kirsten virus transformed NRK cells (ATCC, CRL-1569) were also cultured in a spinner bottle containing the same medium as above.

Preparation of Rat Cultured Cell TMs—A mixture of five TM isoforms were prepared from REF-WT4A cells essentially following the conventional method including heat treatment and ammonium sulfate fractionation (4-7). Cells were cultured in large square plates (245 x 245 x 20 mm, Nunc), washed three times with phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8 mM NaH₂PO₄ (pH 7.3)), and stored at -70°C. Cells in wet weight of 200 g were homogenized with a Waring Blender (three times, 15 s each).
The treatment with Silane A174 is necessary to make gels adhesive. The inner surface had been treated with Silane A174 (Pharmacia). Precipitated in 80% acetone and dissolved in a small volume of 10 mM Tris- HCl, pH 8.0. After incubation for 90 min at room temperature, the mixtures were centrifuged in a Beckman 50.1 Ti rotor (Beckman Instruments) at 200,000 × g for 20 min. TM alone was not precipitated in this condition. Supernatants were carefully separated from pellets, and both fractions were suspended in an equivalent volume of 1 × SDS sample buffer. Samples were run on 12.5% SDS-polyacrylamide gels (12.5% acrylamide, 0.1% bisacrylamide), stained quantitatively with fast green (23), and scanned with a Hoefer densitometer (GS 300) as described previously (11).

**Antibody Production**—Antibodies to the low M, TMs (TM-4 and TM-5) were made by the subcutaneous immunization of rabbits with the protein purified by preparative SDS-gel electrophoresis of crude TM from REF-WT4A cells. For the first injection, 200 μg of protein was emulsified with an equal volume of Freund’s complete adjuvant, and six subsequent boosts were done with 200 μg of protein emulsified in Freund’s incomplete adjuvant over a 5-month period. Seven days after the last injection, serum was collected. Antibodies to chicken gizzard smooth muscle TM were newly raised in a similar way as described previously (11). Preimmune sera and antibodies were adsorbed with nonimmunized nitrocellulose membranes of Western blots (24) by the methods of Olmsted (25) and of Smith and Fisher (26) as described previously (27). The specificities of the antibodies were tested by Western blots and immunofluorescence. Immunoblots showed that the antibody raised only with TM when total cell lysates from REF-52 cells were used. Immunofluorescence staining of cultured cells with these antibodies showed a periodic stain of microfilament bands typical to TM.

**RESULTS**

Separation of TM Isoforms from Rat Cultured Cells—Crude TM was prepared from REF-WT4A cells by the conventional method including heat treatment and subsequent ammonium sulfate fractionation as described in detail under “Materials and Methods.” The purity was estimated to be about 50% by densitometry of 12.5% SDS-polyacrylamide gels stained with Coomassie Brilliant Blue. About 120 mg of crude TM was obtained from 200 g (wet weight) of cells. Final purification and partial separation of the TM isoforms were performed by chromatography on hydroxylapatite (Fig. 1). Crude TM (20 mg) was applied to a hydroxyapatite (Bio-Rad) column (1.2 × 25 cm) pre-equilibrated with 10 mM sodium phosphate buffer (pH 7.4) and incubated overnight. After elution with 80% acetone, the gels were air-dried and fractionated by dissolving in a small volume of 10 mM Tris- HCl, pH 8.0. Acetone precipitations were repeated twice. A mixture of TM-4 and TM-5 was further separated into individual isoforms by preparative isoelectric focusing (described below). After concentrating the supernatant of gels, protein samples were centrifuged in a Beckman 50.1 Ti rotor (Beckman Instruments) at 200,000 × g for 20 min. The resulting pellet was reextracted in 100 ml of high salt solution containing 0.1 mM NaCl, 20 mM imidazole buffer of pH 7.0. After incubation for 90 min at room temperature, the mixtures were centrifuged in a Beckman preparative ultracentrifuge. The supernatants were carefully separated from pellets, and both fractions were suspended in an equivalent volume of 1 × SDS sample buffer. Samples were run on 12.5% SDS-polyacrylamide gels (12.5% acrylamide, 0.1% bisacrylamide), stained quantitatively with fast green (23), and scanned with a Hoefer densitometer (GS 300) as described previously (11).

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**RESULTS**

Separation of TM Isoforms from Rat Cultured Cells—Crude TM was prepared from REF-WT4A cells by the conventional method including heat treatment and subsequent ammonium sulfate fractionation as described in detail under “Materials and Methods.” The purity was estimated to be about 50% by densitometry of 12.5% SDS-polyacrylamide gels stained with Coomassie Brilliant Blue. About 120 mg of crude TM was obtained from 200 g (wet weight) of cells. Final purification and partial separation of the TM isoforms were performed by chromatography on hydroxylapatite (Fig. 1). Crude TM (20 mg) was applied to a hydroxyapatite (Bio-Rad) column (1.2 × 25 cm) pre-equilibrated with 10 mM sodium phosphate buffer (pH 7.4) and incubated overnight. After elution with 80% acetone, the gels were air-dried and fractionated by dissolving in a small volume of 10 mM Tris- HCl, pH 8.0. Acetone precipitations were repeated twice. A mixture of TM-4 and TM-5 was further separated into individual isoforms by preparative isoelectric focusing (described below). After concentrating the supernatant of gels, protein samples were centrifuged in a Beckman 50.1 Ti rotor (Beckman Instruments) at 200,000 × g for 20 min. The resulting pellet was reextracted in 100 ml of high salt solution containing 0.1 mM NaCl, 20 mM imidazole buffer of pH 7.0. After incubation for 90 min at room temperature, the mixtures were centrifuged in a Beckman preparative ultracentrifuge. The supernatants were carefully separated from pellets, and both fractions were suspended in an equivalent volume of 1 × SDS sample buffer. Samples were run on 12.5% SDS-polyacrylamide gels (12.5% acrylamide, 0.1% bisacrylamide), stained quantitatively with fast green (23), and scanned with a Hoefer densitometer (GS 300) as described previously (11).
Multiple Isoforms of Cultured Cell Tropomyosin

About 120 mg of crude TM (a mixture of the five TM isoforms) were applied on a hydroxylapatite column (1.2 x 25 cm) and eluted with a linear phosphate gradient (10–300 mM) containing 5 mM \(\beta\)-mercaptoethanol and 0.1 M KCl (200 ml/chamber). Fractions of 4 ml were collected and column fractions were monitored by SDS-polyacrylamide gel electrophoresis. Fraction numbers are indicated on the top of the electrophoresis pattern. TM-5, TM-4, TM-3, TM-1, and TM-2 were eluted in this order (each TM isoform is indicated by the number). Inset shows isoelectric focusing gel patterns from fraction 49 to fraction 61 (top, basic; bottom, acidic), indicating that TM-5 was eluted faster than TM-4. Lane A, crude TM. Molecular weight markers were indicated on the right of the figure.

**Table I**

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<thead>
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<th>Amino acid composition of tropomyosin isoforms</th>
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**Peptide Analysis**—Because of the similar properties of the buffer (pH 7.0) containing 5 mM \(\beta\)-mercaptoethanol and 0.1 M KCl. After washing with two column volumes of the same solution, proteins were eluted with a linear phosphate gradient (10–300 mM phosphate) containing 5 mM \(\beta\)-mercaptoethanol and 0.1 M KCl (200 ml/chamber). Because the UV absorption of TM is very low, column fractions were monitored by 12.5% SDS-polyacrylamide gel electrophoresis (Fig. 1). Most of the impurities were eluted below about 140 mM phosphate concentration. Above this concentration, TM isoforms were eluted with partial separation. First TM-4/TM-5, then TM-3, TM-1, and TM-2 were eluted. The partial separation of TM-4 and TM-5 was also observed. Isoelectric focusing gel electrophoresis (inset of Fig. 1) revealed that the leading edge contained predominantly TM-5 while the trailing edge contained mostly TM-4. The yield of total amounts of purified TM was about 125 \(\mu\)g/g of wet weight cells.

**Amino Acid Compositions**—Table I shows the amino acid compositions of the TM isoforms purified by preparative electrophoresis and isoelectric focusing (see the section under "Materials and Methods"). The compositions are very similar to each other and also to those of muscle and nonmuscle TM such as rabbit skeletal \(\alpha\)-TM (34) and horse platelet TM (35). The high glycine levels observed in rat cultured cell TM may be due to the contamination caused by preparative electrophoresis although all samples were passed through G25 to remove free glycine. The absence of proline and the high concentrations of charged residues are characteristic of the amino acid composition of TM. The absence of proline is consistent with the previous results that none of the five isoforms of rat cultured cell TM is metabolically labeled with radioactive proline or tryptophan (11).

**Peptide Analysis**—Because of the similar properties of the
five isoforms of TM, they were compared to a peptide level. The purified proteins were cleaved at methionine residues with CNBr under two different conditions (see the section under “Materials and Methods”). TM-1, TM-2, and TM-3 produced similar peptide patterns (Fig. 2). Especially, the peptide pattern of TM-2 was quite similar to that of TM-3 although several different peptides could be recognized in the peptides generated in the milder condition (indicated by arrows in series B of Fig. 2). On the other hand, peptide patterns generated from either TM-4 or TM-5 were significantly different from those of TM-1, TM-2, and TM-3. The peptide maps of TM-4 and TM-5 were again quite similar to each other; however, at least one peptide was found to be clearly different (indicated by the arrows in Fig. 2). These comparative analyses suggest that the rat cultured cell TMs are divided into two structurally related groups: one includes TM-1, TM-2, and TM-3 with high $M_r$ values on SDS gels, and the other includes TM-4 and TM-5 with low $M_r$ values on SDS gels. Although it is known that the mobility of tropomyosin on SDS gels does not correspond to the real $M_r$ (36), we tentatively call TM-1, TM-2, and TM-3 high $M_r$ TMs, and TM-4 and TM-5 low $M_r$ TMs in this paper.

This structural relation is also supported by immunological analysis. We have raised two polyclonal antibodies: one is against chicken gizzard smooth muscle TM, and the other is against rat cultured cell low $M_r$ TMs (TM-4 and TM-5). The cross-reactivities with these two different antibodies were examined by immunoblots using the five isoforms of rat cultured cell TM. Because anti-smooth muscle TM antibody was raised against TM from chicken, we have affinity-purified the antibody from anti-chicken gizzard TM antibody using TM-1-blotted nitrocellulose membrane as an affinity matrix (25-27). As Fig. 3 shows, the affinity-purified antibody to TM-1-cross-reacted strongly with TM-2 and TM-3 but rather weakly with TM-4 or TM-5 (lane 2). On the other hand, anti-TM-4/TM-5 antibody reacted strongly with TM-4 and TM-5, but cross-reacted weakly with TM-1, TM-2, and TM-3 (lane 3). These immunological analyses suggest that the high $M_r$ TMs have the structure similar to smooth muscle TM, and that high and low $M_r$ TMs share a stretch of homologous sequences.

Homodimer Formation—Skeletal muscle $\alpha$- and $\beta$-TMs are known to form $\alpha$-$\alpha$ and $\alpha$-$\beta$ dimers (37). Because two monomers are in register in skeletal muscle TM, the cysteine residue of each monomer is close enough to form an inter-

![Fig. 2. Peptide mapping of the five TM isoforms.](image)

![Fig. 3. Immunological cross-reactivities between the five TM isoforms.](image)
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The monomer composition of cross-linked dimers could then be analyzed by the second dimensional SDS-polyacrylamide gel electrophoresis after the cleavage of disulfide bond with DTT (see the section under “Materials and Methods”).

Oxidation of rat cultured cell TMs yielded three new bands with $M_r$ of 90,000, 85,000, and 65,000 on SDS gels without DTT (Fig. 4A). Concomitantly, all monomer TM bands except the TM-3 band disappeared, although a small amount of TM-4 and TM-5 remained not cross-linked.

The second dimensional analysis in the presence of DTT revealed that the 90-kDa band was composed of only TM-2 and that the 85-kDa band was composed of only TM-1, indicating that both TM-1 and TM-2 are present as homodimers (Fig. 4B). The 65-kDa band was found to be composed of TM-4 and TM-5, but it is not clear whether they form homo- or heterodimers because a large spot of TM-4 covered a small spot of TM-5 (amounts of TM-4 are higher than those of TM-5 in the TM preparation from REF-WT4A cells).

In order to determine this, we have examined dimer formation of TMs of Kirsten virus transformed NRK cells (ATCC 1569). This cell line has previously been shown to contain more TM-5 than TM-4, as well as a small amount of TM-1 (neither TM-2 nor TM-3 was detected in this cell line) (19). Instead of the isolated TM, TM-containing microfilaments (11) were oxidized and analyzed in the same way.

Oxidation of TM-4 and TM-5 gave one triplet band corresponding to cross-linked dimers. Second dimensional gel analysis (Fig. 5A) showed that the upper two bands were composed of TM-5 and the lower band was composed of TM-4. Although it is unknown why oxidized TM-3 gave two bands, the results indicate that both TM-4 and TM-5 form homodimers.

We have also determined if TMs in microfilaments isolated from REF-WT4A cells are present as homodimers as observed for isolated TMs. As Fig. 5B shows, the second dimensional analysis of oxidized TM-containing microfilaments gave the TM pattern similar to that obtained from isolated tropomyosins (Fig. 4B). These experiments indicate that all isoforms of TM except TM-3 are present as homodimers either in isolated forms or in microfilament-bound forms.

Physical Properties—Skeletal muscle TM is known to aggregate in a head-to-tail fashion to form a linear polymer at low ionic strength. On the contrary, nonmuscle TMs with low $M_r$ values isolated from either platelet (38) or brain (39) show a greatly reduced ability for heat-to-tail polymerization. Therefore, we have asked if the five isoforms of rat cultured cell TM differ in their salt-dependent polymerizability.

Using crude TM (a mixture of five TM isoforms), both Stokes radii and sedimentation coefficients of the five TM
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Fig. 5. Analyses of dimer formation of TMs in the isolated microfilaments. A, TM-containing microfilaments isolated from Kirsten virus-transformed NRK cells were oxidized and analyzed in the same way as shown in Fig. 4. Second dimensional gels revealed one spot corresponding to TM-1 (indicated by 1), two spots corresponding to TM-5 (indicated by 5), and one spot corresponding to TM-4 (indicated by 4). These spots are not aligned in the direction of electrophoresis, indicating that TM-1, TM-4, and TM-5 are all present as homodimers. B, TM-containing microfilaments from REF-WT4A cells were analyzed in the same way. Note that the TM pattern is virtually identical to that produced by the isolated TMs (Fig. 4B), suggesting that TMs in microfilaments are also present as homodimers. A spot indicated by a is not a TM and may be a 37-kDa protein which was found in isolated microfilaments but has not yet been identified (11).

### Table II

<table>
<thead>
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<th>Salt conditions</th>
<th>Stokes radius</th>
<th>Sedimentation coefficient</th>
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<td>TM-1, TM-2, and TM-3</td>
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<td>12.3</td>
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<td>TM-1, TM-2, and TM-3</td>
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*Measured by gel filtration on Sephacryl S-300.

Electron Microscopy—The molecular forms of TM isoforms were observed with an electron microscope using the low-angle rotary shadowing technique (28). As Fig. 1 shows, fractions 55, 65, and 75 of the hydroxylapatite column chromatography contained a mixture of TM-4/TM-5, a mixture of TM-3/TM-4, and a mixture of TM-1/TM-2, respectively; these fractions were used for the comparison between high and low M, TMs. When they were observed at a high KCl concentration (0.6 M), all fractions contained rod-like molecules, about 40-nm long (Fig. 6), which is characteristic of tropomyosin. We have also examined TMs of fractions 51 and 61 of the hydroxylapatite column, which contained mostly TM-5 and TM-4, respectively (see inset of Fig. 1). Similar rod-like molecules were observed in both specimens.

When TMs were observed at a low KCl concentration (0.1 M), fraction 75 of high M, TMs frequently showed longer linear aggregates than fraction 55 of low M, TMs. Although the length of the molecules distributed rather broadly, the average lengths were 130 ± 100 nm for high M, TMs and 82 ± 50 nm for low M, TMs. These observations support the physical properties that high M, TMs polymerize to a higher extent than low M, TMs.

Actin Binding Property—Actin-binding affinities of rat cultured cell TMs were examined using a mixture of five TM isoforms. As Fig. 7A shows, high M, TMs bound to actin more strongly than low M, TMs. To compare the binding affinities between high and low M, TMs, we have also examined actin binding of separated TMs using fraction 75 (TM-1+TM-2) and fraction 55 (TM-4/TM-5) of the hydroxylapatite column. As Fig. 7B shows, the actin binding affinity of high M, TMs (TM-1+TM-2) was again higher than that of low M, TMs.

### Table I

<table>
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<th>Physical properties of tropomyosin isoforms</th>
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<tr>
<td>Salt conditions</td>
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*Measured by gel filtration on Sephacryl S-300.

*Measured by sucrose density gradient centrifugation.

isoforms, at high and low ionic strength, were measured by gel filtration and by sucrose density gradient centrifugation, respectively. Fractions of gel filtration and of density gradient centrifugation were monitored by SDS-gel electrophoresis and isoelectric focusing to determine the positions of each TM isoform. As Table II shows, the results again indicate that rat cultured cell TMs can be classified into two groups, i.e. high M, TMs (TM-1, TM-2, and TM-3) and low M, TMs (TM-4 and TM-5). TM-4 and TM-5 showed a lesser extent of salt-dependent polymerization than TM-1, TM-2, and TM-3. The Stokes radii of TM-1, TM-2, and TM-3 increased from 7.5 to 12.3 nm when NaCl concentration was lowered from 0.6 to 0.1 M. On the other hand, TM-4 and TM-5 changed their Stokes radii from 6.2 to 8.2 nm under the same conditions. The sedimentation coefficients of TM-1, TM-2, and TM-3 increased from 3.4 to 3.7 with decreasing NaCl concentrations from 0.6 to 0.1 M while those of TM-4 and TM-5 remained unchanged.

From the Stokes radii and sedimentation coefficients at 0.6 M NaCl, we have calculated native M, values to be 105,000 for the high M, TMs and 87,000 for the low M, TMs. The values, however, may not be accurate probably because of some interaction between TMs.
FIG. 6. Electron micrographs of TM isoforms separated by the hydroxylapatite column chromatography (Fig. 1). A, fraction 57 (a mixture of TM-4/TM-5); B, fraction 65 (a mixture of TM-3/TM-4); and C, fraction 75 (a mixture of TM-1/TM-2) were observed by the low-angle rotary shadowing technique (28). Rod-like molecules are seen in all specimens but aggregated, longer polymers (indicated by a double arrow in C) are frequently observed in the specimen of high M, TMs. (TM-4 + TM-5). The relative binding ability of TM-1 and TM-2 was found to be same by densitometry of SDS gels. Although TM-4 and TM-5 were not distinguishable on SDS gels, isoelectric focusing revealed that the relative binding appeared to be the same.

The molar ratios of actin to one tropomyosin dimer at saturation were calculated to be 5–6 for both types of TM, assuming that M, values of actin, high M, TMs, and low M, TMs are 42,000, 68,000, and 60,000, respectively. The values are close to but lower than those expected (7 for muscle TM and 6 for nonmuscle TM). The reason for this difference is currently unknown but may be ascribed to experimental errors.

It has been reported that platelet and brain TM needs 5–8 mM Mg2+ to show actin-binding at either 100 or 30 mM KCl (7, 40). We have also examined the effects of Mg2+. At 100 mM KCl, Mg2+ up to 10 mM showed no effects on the actin binding of any isoforms. At 30 mM KCl, however, 5 mM Mg2+ was necessary for all TM isoforms to show the binding. This Mg2+ dependency is similar to that reported for muscle (41), bovine thyroid (18), and human erythrocyte (17) TMs but different from that of other nonmuscle TMs (7, 40, 42).

DISCUSSION

The five isoforms of rat cultured cell TM share many properties, characteristic of TM, including their stability to heat (11), similar isoelectric points (11) and amino acid compositions, and copurification during ammonium sulfate fractionation and DEAE-cellulose column chromatography. The structural (Fig. 2), immunological (Fig. 3), biophysical (Table II), and actin-binding (Fig. 7) analyses, however, indicate that five isoforms fall into two groups: one is TMs (TM-1, TM-2, and TM-3) with high M, values on SDS gels, and the other is TMs (TM-4 and TM-5) with low M, values on SDS gels.

The studies (4–7, 40, 42; see Ref. 43 for review) of TM isolated from nonmuscle tissues such as platelets and brain have revealed several major contrasts between muscle and nonmuscle TMs. First, these nonmuscle tissues were shown to contain only or mainly TMs with lower M, values on SDS gels (M, ~30,000 as compared to ~35,000 of muscle TMs) and with shorter periodicity of Mg-paracrystals (35 nm as compared to 41 nm). The smaller size of the nonmuscle TMs
was directly proven by the complete amino acid sequence where equine platelet TM has a Mr of 28,652 (35) while rabbit skeletal muscle TM has a Mr of 32,800 (34). Second, the sequence comparison has also revealed the marked differences in both N- and C-terminal sequences between muscle and equine platelet TMs. These differences explain another major contrast. Nonmuscle TM shows less ability for head-to-tail polymerization and less affinity to actin (4–7, 40). It is reported that N- or C-terminal sequences are critical for head-to-tail polymerization for skeletal muscle TM and that the ability of TM to undergo head-to-tail polymerization is closely correlated to the ability of its actin-binding (43–45). When skeletal muscle TM is treated with carboxypeptidase to remove several amino acids from the C-terminal end, the digested TM loses its polymizability and actin-binding ability simultaneously (44).

Similar contrasts are also observed between high (TM-1, TM-2, and TM-3) and low (TM-4 and TM-5) Mr TMs from rat cultured cells. TM-1, TM-2, and TM-3 showed mobilities similar to those of skeletal, smooth, or cardiac muscle TM, while the mobilities of TM-4 and TM-5 are close to that of platelet TM on SDS gels. Although it is known that TM shows anomalous mobility on SDS gels, differences in Mr may be involved in these cases judging from the studies described below. The amino acid sequence of TM-1 derived from cDNA clone has revealed that TM-1 contains 284 amino acids (the same chain length as muscle TM) whose sequence is very similar to that of chicken gizzard α-TM (46). In addition, Mg-paracrystals made with TM-4 and TM-5 show the shorter periodicity (35 nm) indicating that TM-4 and TM-5 are indeed low Mr TMs. The low Mr TMs showed less ability for head-to-tail polymerization (Table II) and less affinity for actin (Fig. 7) than the high Mr TMs. Thus, one may suggest that high Mr TMs (TM-1, TM-2, and TM-3) may be muscle forms of TM resulting from some alterations in gene expression.

Acknowledgments—We are grateful to Dr. J. D. Watson for his support. We thank Joseph Sohns for helping with electron microscopy, Midori Maruyama for her technical assistance, Phil Renna for his photographic skills, and Madeline Szadkowski for typing the manuscript.

REFERENCES
Multiple Isoforms of Cultured Cell Tropomyosin