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# Tropomyosin Heterogeneity in Human Cells*

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**Tropomyosin preparations from human platelets, human peripheral blood leukocytes from normal individuals and from a patient with chronic lymphocytic leukemia, human lymphoblastoid cells (GM607), human epithelial cells, and human skin fibroblasts have all been found to contain more than one protein when analyzed by two-dimensional gel electrophoresis. Although the lymphoid cell preparations consistently contain two proteins of almost identical molecular weight ($M_r$ = 30,000), the platelet, epithelial cell, and fibroblast preparations contain two or more major proteins with molecular weights between 31,000 and 36,000, in addition to a major protein at 30,000. All of these proteins have characteristics in common with tropomyosin including slightly acidic isoelectric point (approximately pH 4), stability to heat and organic solvents, association with the cytoskeleton, and reactivity with antibody against skeletal muscle tropomyosin. The nonmuscle tropomyosin-like proteins were compared with tropomyosins from human skeletal, cardiac, and smooth muscle by peptide mapping after partial proteolysis. The results showed one of the nonmuscle proteins to be identical to the major smooth muscle tropomyosin in human uterus (myometrium) and another to be similar but not identical to skeletal muscle $\alpha$-tropomyosin. The remainder of the proteins with tropomyosin characteristics was unique to nonmuscle cells. In all, nine distinct human proteins with characteristics of tropomyosin are described. Charge variants of two of these proteins have been described previously.**

Tropomyosin preparations from a variety of nonmuscle sources including bovine brain (1–3), human, equine, and porcine platelets (4–6), mouse fibroblasts (2, 7), baby hamster kidney (7), and rat embryo (7) have been reported to contain either one or two major proteins with an SDS[1] molecular weight of approximately 30,000. Minor proteins with slightly higher molecular weights were also present in some of these preparations, but little or no characterization of the minor proteins has been reported. The high-molecular-weight components of the brain tropomyosin preparations were thought to be smooth muscle tropomyosin from vascular tissue that could not be completely removed from the brain tissue used as starting material (2, 3). The minor proteins in the baby hamster kidney and rat embryo cell preparations co-migrated with muscle tropomyosin on one-dimensional SDS-PAGE, but no further characterization was reported (7). Using two-dimensional electrophoresis, Garrels found that prefusion myoblasts contain three tropomyosin molecules, two nonmuscle and one smooth muscle (8). More recently, analyses of tropomyosin-enriched preparations from rat embryo fibroblasts (9, 10), rat kidney cells (9), human skin fibroblasts (11–13), and chicken embryo fibroblasts (14) by two-dimensional electrophoresis have shown that different cells contain between 3 and 7 proteins with tropomyosin-like characteristics, several of them with molecular weights above 30,000. These results indicate that there may be co-expression of more than one type of tropomyosin within a single cell.

We have described charge variants of two human fibroblast proteins that both have tropomyosin-like characteristics (11, 12). One of the fibroblast proteins has a molecular weight of approximately 30,000 and has also been found in human peripheral blood leukocytes and a lymphoblastoid cell line, GM607 (11, 15). The other protein has a molecular weight of approximately 35,000 and has a cleavage pattern after partial proteolysis that is identical to the major tropomyosin-like protein from human uterus (myometrium), a smooth muscle (12). In addition to these two proteins, human fibroblasts have four other proteins with tropomyosin-like characteristics. Comparison of the fibroblast proteins with those isolated from human platelets, epithelial cells, lymphoid cells, skeletal muscle, cardiac muscle, and smooth muscle (uterus) by tropomyosin purification techniques (1) has revealed a set of nine proteins that are differently expressed in the different tissues analyzed. Skeletal muscle $\alpha$-tropomyosin is also found in cardiac muscle, while $\beta$-tropomyosin seems to be restricted to skeletal muscle. Uterine tropomyosin preparations have several proteins in common with similar preparations from platelets, epithelial cells, lymphoid cells, and fibroblasts. These results suggest that multiple tropomyosin genes exist and that more than one such gene can be expressed in a single cell type. The differences in tropomyosin expression may be related to functional differences in the cytoskeletons of nonmuscle cells.

## MATERIALS AND METHODS

*Sample Acquisition*—Samples of normal human skeletal muscle were provided by Dr. M. J. Danon (University of Illinois Hospital, Chicago, IL); human cardiac muscle was obtained from Dr. J. Devine (St. Louis Hospital, St. Louis, MO); and human smooth muscle (myometrium) and blood samples from which platelets and leukemic cells were isolated were provided by Dr. L. Lonzer (Hinsdale Sanitarium Hospital, Hinsdale, IL). GM607 was from the Human Genetic Mutant Cell Repository (Institute for Medical Research, Camden, NJ). Human skin fibroblasts (cell line 1494) were from Meloy Laboratories (Springfield, VA) under National Cancer Institute Contract N01-CP91000. Human epithelial cells (CCL 239; normal colon), promyelocytic leukemia cells (HL60), and histiocytic lymphoma cells (CRL 1593; U937) were obtained from the American Type Culture Collection (Rockville, MD).

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[1] The abbreviations used are: SDS, sodium dodecyl sulfate; PBL, peripheral blood leukocyte(s); pI, isoelectric point; PAGE, polyacrylamide gel electrophoresis; Tm, tropomyosin; NP40, Nonidet P-40.

*Preparation of Tissue Samples for Electrophoresis*—Skeletal, cardiac, and smooth muscle samples were all frozen and stored at −70 °C. Tissue was pounded into a frozen powder with a stainless steel mortar and pestle chilled with dry ice. The frozen powder was diluted with NP40-urea mix (9 M urea, 4% Nonidet P-40, 2% ampholytes (LKB pH 9–11), 2% 2-mercaptoethanol), 10 μl/mg, frozen tissue weight, and then centrifuged for 3 min in a Beckman model B microfuge. The supernatants were subsequently centrifuged for 1 h at 40,000 rpm in a Beckman 42.2 rotor. Aliquots of the resulting supernatants were analyzed by two-dimensional electrophoresis.

*Preparation of Platelets for Electrophoresis*—Platelets were obtained by differential centrifugation of human blood that was collected for leukophoresis. Final preparations contained approximately $10^6$ platelets/ml with no detectable leukocytes or erythrocytes (counted both manually and mechanically (Coulter model 5, modified ZBI)). Platelet pellets were solubilized in NP40-urea mix, 5 ml/ml of packed cells.

*Preparation of Cultured Cells for Electrophoresis*—PBL from clinically normal individuals were isolated by Ficoll-Hypaque gradient centrifugation as described previously (16). GM607, PBL, HL60, U937, CCL239, and 1494 were all radiolabeled by culturing for 18 h in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) minus methionine containing 50 μCi/ml of [$^{35}$S]methionine (1150 Ci/mmol), with approximately $10^6$ cells/well in flat-bottomed multiwell plates (Falcon 3047). Cells were harvested for two-dimensional gel electrophoresis with 50 μl/well of NP40-urea mix. For tropomyosin preparations, the cells were taken up in absolute ethanol and mixed with 1.0 g (wet weight) of human platelets as a nonradiolabeled protein carrier.

*Tropomyosin Preparations*—Tropomyosin was prepared from human skeletal muscle, cardiac muscle, smooth muscle, platelets, lymphoid cells, epithelial cells, and fibroblasts by the technique outlined by Fine and co-workers (1) through the 53% ammonium sulfate precipitation. Sufficient tropomyosin was obtained for this study with 1.0 g (frozen weight) of skeletal muscle (flexor), 2.0 g (wet weight) of cardiac muscle, 1.0 g (wet weight) of uterus, and 1.0 g (wet weight) of platelets. As much as 10 g of platelets and 55 g of lymphocytes (from a patient with chronic lymphocytic leukemia) were used for some experiments. PBL, GM607, CCL239, and 1494 cells were all labeled with [$^{35}$S]methionine prior to isolation of tropomyosin, and human platelets (1.0 g) or lymphocytes (1.0 g) were used as nonradiolabeled protein carrier. After the 53% ammonium sulfate precipitation, the precipitates were dissolved in 0.05 M Tris, pH 8.0, and dialyzed against double-distilled water overnight. The samples were then lyophilized, dissolved in NP40-urea mix, and analyzed by two-dimensional gel electrophoresis.

*Two-dimensional Gel Electrophoresis*—Isoelectric focusing was performed as described previously (17) with 10% LKB pH 2.5–4 and 90% LKB pH 3-10 ampholytes. After equilibration with SDS (18), the proteins were separated in the second dimension on 9–18% linear polyacrylamide gel gradients as described (19). Conditions of gel electrophoresis, staining, destaining, and photography were all as described (20). Dried gels were autoradiographed or fluorographed (21) with Kodak XAR-2 film.

*Partial Proteolysis*—To determine the relationship of protein spots in two-dimensional gel electrophoresis patterns of different samples, spots were cored from the fixed, stained, and destained gels and electrophoresed in the SDS-PAGE system described by Cleveland (22). *Staphylococcus* V8 protease or chymotrypsin overlaid the cored-out gel spots as described (11), so that proteolysis occurred as electrophoresis progressed. The final products of this partial proteolysis were stained with silver (23) or Coomassie Blue (20).

*Nitrocellulose Transfer and Staining*—Proteins from homogenates of skeletal muscle, cardiac muscle, smooth muscle, fibroblasts, platelets, and cell line GM607 were transferred from two-dimensional electrophoresis gels onto sheets of nitrocellulose (Schleicher and Schuell, Inc.) as described (24). The nitrocellulose sheets were blocked with 3% bovine serum albumin and then incubated with rat antiserum to chicken leg muscle tropomyosin (a gift from A. MacLeod, Ludwig Institute for Cancer Research, Cambridge, England) as described (12). The protein spots with bound antibody were detected by incubation with peroxidase-conjugated, anti-rat IgG from rabbit (Miles Laboratories), followed by staining in horseradish peroxidase reagent containing 4-chloro-1-naphthol (Bio-Rad).

## RESULTS

Fig. 1 shows portions of the two-dimensional electrophoresis patterns of proteins from human skeletal muscle (quadriceps), cardiac muscle, smooth muscle (myometrium), platelets, fibroblasts (skin), epithelial cells (colon), lymphoblastoid cells (GM607), and cells from promyelocytic leukemia (HL60) and histiocytic lymphoma (U937). The *Tm* numbers identify the proteins that are enriched by tropomyosin preparation, as shown in Fig. 2; proteins with identical *Tm* numbers in different patterns are known to co-migrate (see Fig. 7) and have been characterized as the same protein by partial proteolysis (see below). All of the *Tm* proteins have isoelectric points near 4.1 and SDS molecular weights between 29,500 and 36,000 and have been observed to shift to a higher apparent molecular weight when SDS-PAGE was carried out in the presence of 4 M urea (8, 11), a characteristic of tropomyosin (25). The *Tm* protein pattern from cultured cells is identical in autoradiographs of radiolabeled proteins and in gels containing sufficient unlabeled protein to be detected by Coomassie Blue staining (data not shown).

Fig. 2 is a composite of the two-dimensional gel electrophoresis patterns of tropomyosin preparations from human skeletal muscle, cardiac muscle, smooth muscle, platelets, fibroblasts, and leukocytes (PBL from a patient with chronic lymphocytic leukemia; identical patterns were obtained with radiolabeled normal PBL and GM607). Preparations from epithelial cells showed patterns similar to those of fibroblast proteins (data not shown).

Proteins corresponding in SDS molecular weight to rabbit muscle α- and β-tropomyosins (labeled Tm:1 and Tm:2, respectively, in Figs. 1 and 2) were the most abundant components of the skeletal muscle tropomyosin preparation, with the consistent co-purification of four minor proteins. The protein designated Tm:1′ has a peptide cleavage pattern similar to that of Tm:1 and is thought to be a specialized form of α-tropomyosin associated with the Type I (slow) fibers of human skeletal muscle.[2] Each of the acidic satellite spots beside the three basic proteins (Tm:1, :1′, and :2) in the skeletal muscle tropomyosin preparation has a peptide cleavage pattern identical to that of the respective basic protein. In addition, the acidic protein spots disappear after incubation of either muscle homogenates or skeletal muscle tropomyosin preparations with *Escherichia coli* alkaline phosphatase.[2] These results suggest that the satellite proteins are actually phosphorylated forms of the basic proteins.

Tropomyosin preparations from cardiac muscle contained only two major proteins that co-migrated with Tm:1 and the acidic spot to the left of Tm:1 from skeletal muscle. Only a trace of protein was found in a position comparable to Tm:2 (β-tropomyosin), and no further characterization was done. Identical results were obtained from three separate preparations, including a comparison of tissue from the right and left ventricles of a single individual.

Tropomyosin preparations from human myometrium contained two major proteins (Tm:3 and Tm:6, Figs. 1*C* and 2*C*), both with molecular weights of approximately 35,000 but differing in isoelectric point. Tm:7 (a shoulder to the left of Tm:6) and :8 are also visible in Fig. 2*C*. A minor protein to the right of Tm:3 has no counterpart in the whole protein pattern (Fig. 1*C*), indicating that it could be a fragment of some higher-molecular-weight protein that was degraded by the conditions used for the tropomyosin purification. Tm:4 and :5 were visible on the gels but were too faintly stained to be seen in this photograph.

[2] C. S. Giometti and M. J. Danon, manuscript in preparation.

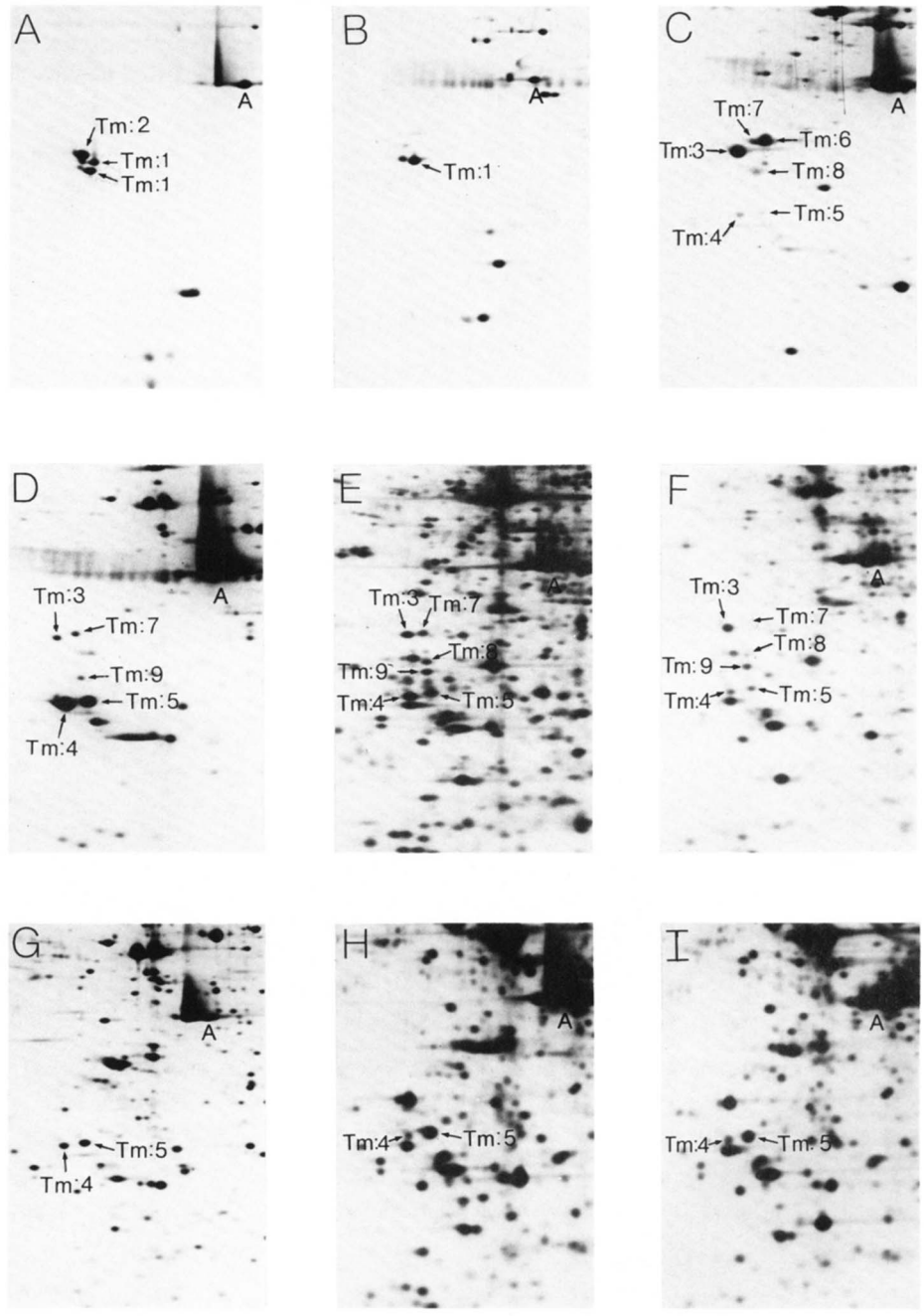


FIG. 1. **Portions of two-dimensional electrophoresis patterns of proteins.** *A*, human quadriceps (skeletal muscle); *B*, human heart (cardiac muscle); *C*, human myometrium (smooth muscle); *D*, human platelets; *E*, human skin fibroblasts (1494); *F*, human epithelial cells (CCL239); *G*, human lymphocytes (GM607); *H*, promyelocytic leukemia (HL60); *I*, histiocytic lymphoma (U937). Sample preparation and electrophoresis were as described under "Materials and Methods." Proteins were detected by Coomassie Blue staining (*A*, *B*, *C*, *D*), autoradiography (*E*, *F*, *G*), or fluorography (*H*, *I*). Gels are oriented with the acidic side to the left and the basic side to the right. *A*, actin ($M_r$ = 43,500); *Tm*, proteins with tropomyosin-like characteristics ($M_r$ = 29,000–36,000). The chain of protein spots to the left of actin in panels *B*, *C*, and *D* are carbamylated creatine kinase standards (26).

Platelet tropomyosin preparations contained Tm:3, :4, :7, and a small amount of :9 (Fig. 2*D*), and their abundances were comparable to those seen in the whole homogenate pattern (Fig. 1*D*). Although Tm:5 was present in the whole platelet samples (Fig. 1*D*), little if any of this protein was isolated in tropomyosin preparations. Several minor proteins are clustered in the region of Tm:5, but none of these proteins had peptide cleavage patterns similar to the platelet Tm:5 found in whole homogenates (data not shown). The minor proteins may be peptide fragments from higher-molecular-weight proteins, since there are no comparable protein spots in Fig. 1*D*. As reported previously, human skin fibroblast tropomyosin preparations contained four proteins (Tm:3, :4, :8, and :9) when platelets (12) or brain (11) were used as carrier protein. When lymphoid cells were used as unlabeled carrier protein, however, Tm:5 and :7 were isolated in addition to Tm:3, :4, :8, and :9 (Fig. 2*D*). Leukocyte tropomyosin preparations consistently contained only two major proteins, Tm:4 and Tm:5 (Fig. 2*E*).

Proteins from whole homogenates of skeletal muscle, cardiac muscle, smooth muscle, platelets, fibroblasts, and leukocytes were transferred from polyacrylamide gels to nitrocellulose and stained with rat antiserum against chicken skeletal muscle tropomyosin. Fig. 3 shows that the proteins binding the anti-tropomyosin were comparable to those in tropomyosin preparations (Fig. 2) with two exceptions. Tm:5 was barely visible after reaction with the antiserum even though it was almost equal in abundance to Tm:4 in whole homogenates (Fig. 1). Note that Tm:5 in the platelet homogenate did react with the antiserum although that protein does not co-purify with other platelet *Tm* proteins. Secondly, Tm:7 was visible as a shoulder to the left of Tm:6 in the pattern of uterus proteins after reaction with the anti-tropomyosin (Fig. 3*C*) but was not detected at all in the platelet (Fig. 3*D*) or

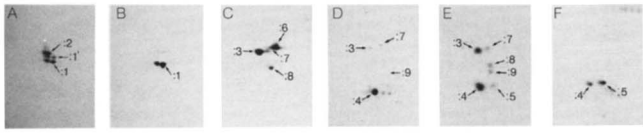


FIG. 2. **Two-dimensional electrophoresis of tropomyosin preparations.** *A*, human skeletal muscle; *B*, human cardiac muscle; *C*, human smooth muscle; *D*, human platelets; *E*, human skin fibroblasts; *F*, human leukocytes. Samples were prepared as described under "Materials and Methods." Only the portion of the gel containing tropomyosin is shown; the remainder of the gel contained no protein. Carbamylated creatine kinase, used as a charge standard (26), can be seen in the *upper right corner* of *panel E*. Proteins were detected by Coomassie Blue staining (*A*, *B*, *C* and *F*) or fluorography (*E*). Gel orientations are as in Fig. 1. *Tm* proteins are indicated by *number*.

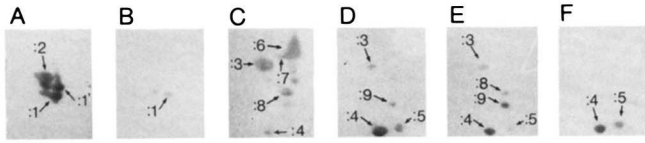


FIG. 3. **Proteins that react with rat antiserum to chicken skeletal muscle tropomyosin.** Proteins were transferred from two-dimensional electrophoresis gels onto nitrocellulose and treated with anti-tropomyosin. The proteins that bound the antibody were detected with anti-rat IgG-peroxidase conjugate followed by reaction with horseradish peroxidase reagent. *A*, skeletal muscle; *B*, cardiac muscle; *C*, smooth muscle; *D*, platelets; *E*, skin fibroblasts; *F*, leukocytes (GM607). Patterns are oriented as described for Fig. 1; *Tm* proteins are indicated by *number*.

fibroblast (Fig. 3*E*) protein patterns although Tm:3 was detected and Tm:3 and :7 were present in the whole homogenate patterns (Fig. 1, *D* and *E*) in approximately equal abundance. Several protein spots directly under Tm:6 in the uterus pattern also reacted with the antiserum. These proteins were also visible in the whole homogenate pattern (Fig. 1*C*) but were not found in the uterus tropomyosin preparations (Fig. 2*C*). Their absence from the tropomyosin preparations suggests that these proteins are smaller forms of Tm:6, perhaps proteolytic fragments or peptides formed by intramolecular disulfide bonding due to insufficient reduction, that are lost during the tropomyosin purification procedure.

To further investigate the relationship of the tropomyosin proteins in different cell and tissue types, the protease digestion products after partial proteolysis of the 10 proteins (Tm:1–Tm:9 plus Tm:$1_c$) were compared. Although one-dimensional electrophoresis patterns of peptide fragments do not guarantee that the proteins compared have identical amino acid sequences, this method does show that certain proteins are totally unrelated (*i.e.* have distinct cleavage patterns) while others are clearly similar in their primary structure. The similarity of two proteins can be further ensured by comparing the results of cleavage by enzymes with different specificities, such as chymotrypsin which cleaves peptide bonds at the carboxyl end of aromatic amino acids *versus* *Staphylococcus* V8 protease which breaks peptide bonds at the carboxyl end of aspartic and glutamic acid residues. Tropomyosin spots were cut from gels and digested with either chymotrypsin or *Staphylococcus* V8 protease, and the digestion products were separated by one-dimensional SDS electrophoresis. Fig. 4 compares the products obtained from the 9 different *Tm* proteins plus the major cardiac protein thought to be tropomyosin. Tm:1 from skeletal muscle produced a pattern identical to that of the cardiac muscle protein (termed Tm:$1_c$). Tm:1 and Tm:2 ($\alpha$- and $\beta$-tropomyosin, respectively) from skeletal muscle had distinct cleavage patterns. Tm:3 had a cleavage pattern similar to Tm:2 when V8 protease was used but a very distinct pattern after cleavage with chymotrypsin. Tm:4 through Tm:7 produced very different patterns with the two enzymes used. Tm:8 had similar patterns to Tm:9 after cleavage with V8 protease, suggesting that those two proteins have similar arrangements of aspartic and glutamic acid residues. Cleavage with chymotrypsin, however, revealed two different sets of peptides for Tm:8 and :9, demonstrating their lack of identity.

The cleavage patterns of Tm:8 were so similar to those of Tm:1 that identity was suspected. A peptide that was present in the Tm:1 cleavage pattern after digestion with chymotrypsin appeared to be missing or at least present in a smaller amount in the Tm:8 cleavage pattern (see *arrows* in Fig. 4*C*; note that the peptide above the *arrow* in the Tm:1 pattern is almost equal in abundance to the peptide beside the *arrow*, while in the Tm:8 pattern the peptide above the *arrow* is visible but the one beside the *arrow* is not). In order to determine more conclusively whether or not Tm:1 and :8 were the same or different proteins, a co-migration study was done. If two protein samples are truly identical, co-migration of a radiolabeled sample with a nonradiolabeled sample will result in the dilution of the radiolabeled protein so that a diffuse spot is seen on the final autoradiograph rather than the usual

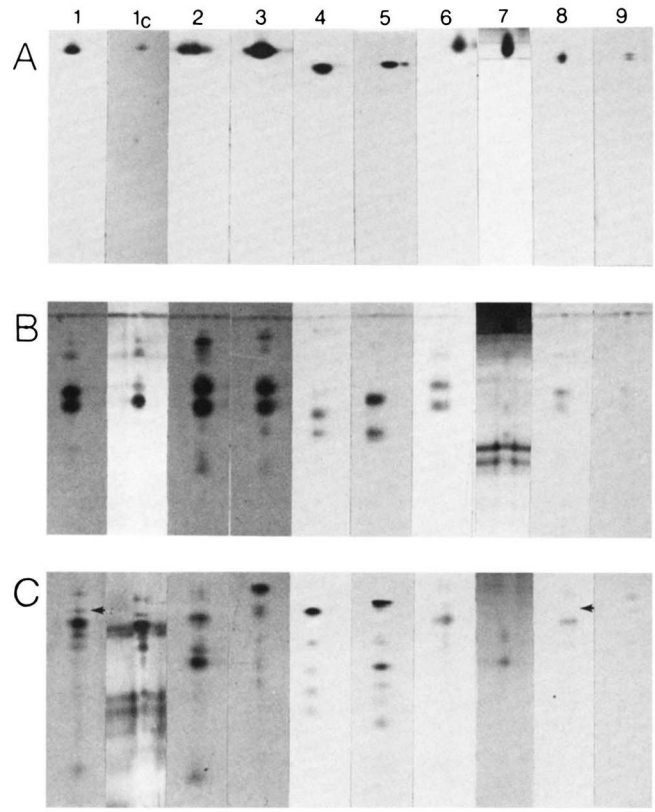


FIG. 4. **Peptide fragments from partial proteolysis of *Tm* proteins; comparison of the nine different proteins.** Proteolysis and electrophoresis were as described under "Materials and Methods." *Numbers at the top* of the figure correspond to *Tm* proteins 1 through 9 (plus $1_c$ from cardiac muscle). *A*, control without protease; *B*, peptides after digestion with *Staphylococcus* V8 protease; *C*, peptides after digestion with chymotrypsin. Peptides were detected by silver (7A, $1_c$B, 7B, $1_c$C, 7C) or Coomassie Blue staining. The more sensitive silver staining revealed protein bands corresponding to the protease used as well as the sample peptides (*e.g. panel* $1_c$*C*). These protease bands extend across the gels and serve as internal standards for the comparison of peptide fragments on the same and different gels. *Arrows* in *panel C* indicate peptide differences in Tm:1 and :8.

tight dark spot (18). Co-electrophoresis of [$^{35}$S]methionine-labeled fibroblast proteins (containing Tm:8) with unlabeled proteins from skeletal (containing Tm:1) and smooth muscle (containing Tm:8) showed that the fibroblast $^{35}$S-labeled Tm:8 was diluted when mixed with the smooth muscle proteins but was pushed to the side when mixed with the skeletal muscle proteins (Fig. 5). These results indicate that fibroblast Tm:8 is different from skeletal muscle Tm:1 but identical to smooth muscle Tm:8. Additional proof of the uniqueness of Tm:1 and Tm:8 was provided by co-migration of skeletal

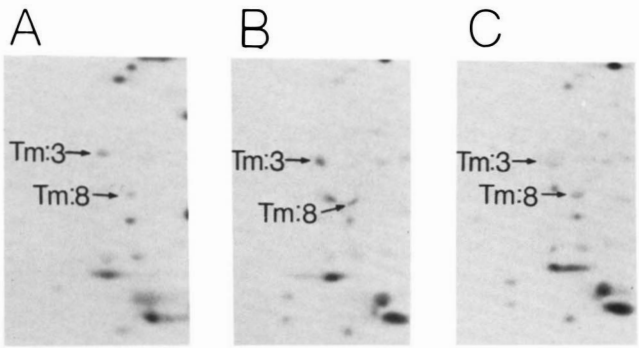


FIG. 5. **Two-dimensional electrophoresis patterns of fibroblast proteins labeled with [$^{35}$S]methionine after co-electrophoresis with muscle proteins.** *A*, human skin fibroblasts ([$^{35}$S]methionine) plus human skin fibroblasts (unlabeled); *B*, human skin fibroblasts ([$^{35}$S]methionine) plus skeletal muscle (unlabeled); *C*, human skin fibroblasts ([$^{35}$S]methionine) plus smooth muscle (unlabeled). Proteins were detected by autoradiography. Labels and gel orientations are as in Fig. 1. Data show that Tm:1 and :8 are distinct proteins.

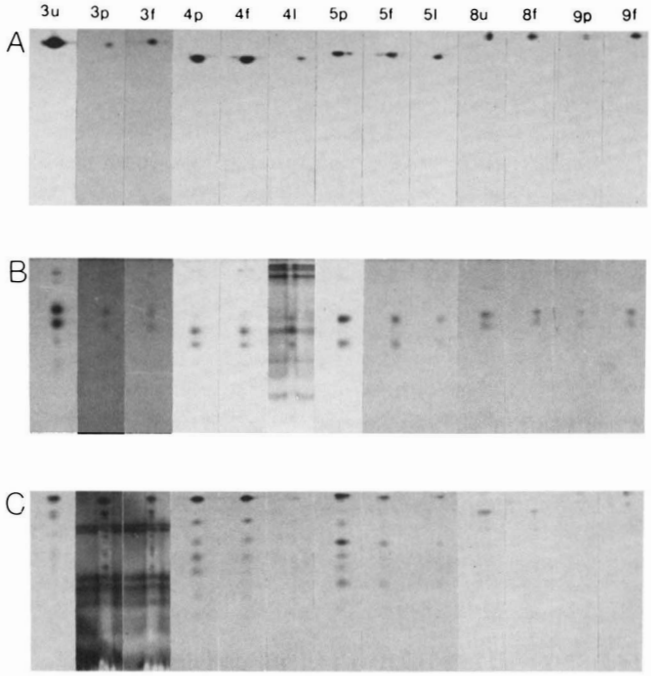


FIG. 6. **Peptide fragments after partial proteolysis of *Tm* proteins from different tissue and cell types.** The numbers refer to the respective *Tm* proteins. Tm:6 is not included because it was found only in smooth muscle. Tm:7 cleavage products were only faintly stained even by silver, so these data are not included; visible bands were identical when Tm:7 from platelets, uterus, and fibroblasts were compared. *u*, uterus (myometrium); *p*, platelet; *f*, fibroblast; *l*, lymphocyte; *A*, control without protease; *B*, peptides after digestion with *Staphylococcus* V8 protease; *C*, peptides after proteolysis with chymotrypsin. Peptides were detected by either silver (*4lB*, *3pC*, *3fC*) or Coomassie Blue staining as described for Fig. 4.

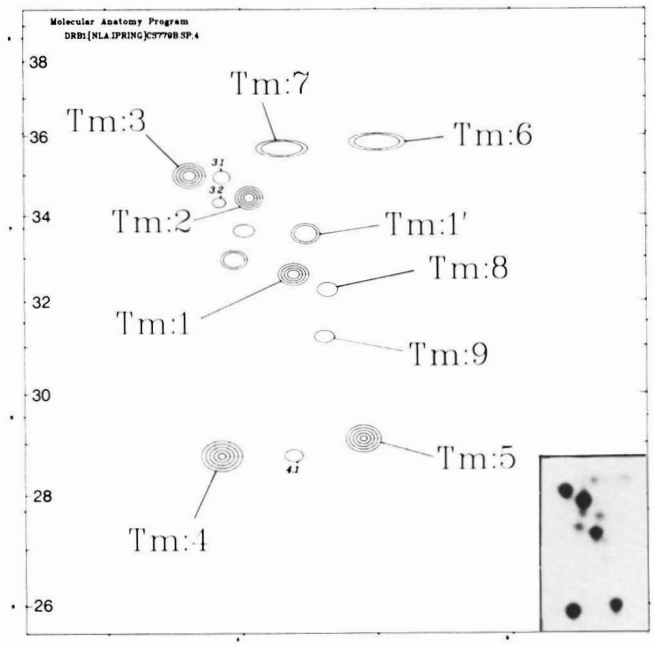


FIG. 7. **Composite diagram of the *Tm* protein set.** Samples of human skeletal muscle, smooth muscle, and platelets were mixed and the proteins separated by two-dimensional gel electrophoresis as described under "Materials and Methods," except that 60% Pharmalyte pH 4–6.5, 20% Pharmalyte pH 3–10, and 20% Servalyte pH 3–10 were used for the first dimension separation and 12% slab gels were used for the second dimension separation. The diagram was generated after image processing of the silver stained gel (*inset* in *lower right corner* of diagram shows actual gel pattern) using the TYCHO image analysis system (27) and represents the positions of the nine *Tm* proteins on the gels. *Numbers* on the *x* axis are positional values only; molecular weights (*y* axis) were obtained by using human serum proteins as a standard (28). Numbers within the diagram correspond to *Tm* protein numbers. *Small circles* marked *3.1* and *4.1* show locations of tropomyosin charge variants described elsewhere (11, 12); *small circle* marked *3.2* is another charge variant of Tm:3 that is presently under investigation (29).

muscle, smooth muscle, and platelet proteins to obtain the composite pattern used for Fig. 7. This pattern was obtained using a more expanded pH gradient for the first-dimension separation (60% pH 4–6.5 with 40% pH 3–10 instead of the usual 10% 2.5–4 with 90% 3–10) and a 12% acrylamide slab instead of 9–18% linear acrylamide gradient for the second-dimension separation, resulting in a more distinct separation of Tm:1 and :8 than seen in the other figures.

Comparison of the peptides from the various tropomyosin proteins of different cells/tissues showed that Tm:3 from uterus, platelets, and fibroblasts had identical cleavage patterns regardless of the protease used (Fig. 6). The same was true for Tm:4, :5, :8, and :9 from different cell types.

Fig. 7 is a composite of the tropomyosin-like proteins from skeletal muscle, smooth muscle, and platelets; Table I summarizes the distribution of the different *Tm* proteins. These proteins comprise a small population with very similar molecular weights (varying by only approximately 5000) and isoelectric points (within 5 charge shift units). Tm:3.1 and :3.2 have been described previously as charge variants (*i.e.* differing in pI and, therefore, assumed to contain an amino acid substitution) of Tm:3 in human skin fibroblasts (12, 29), and Tm:4.1 was found to be a charge variant of Tm:4 (11). Each of these variant proteins was identified by co-purification with the wild-type protein and identical Cleveland peptide patterns, together with an approximately 50% decrease in the abundance of the wild-type protein spot (*e.g.* Tm:4) with the

TABLE I

*Distribution of Tm proteins in different cell types*

This table was formulated from the data in Fig. 1. A minus sign indicates the total absence of the protein, while the number of plus signs indicates the relative quantity of a protein in a particular cell type.

| Sample | *Tm* number | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 1′ | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Skeletal muscle | +++ | +++ | +++ | − | − | − | − | − | − | − |
| Cardiac muscle | +++ | − | − | − | − | − | − | − | − | − |
| *Smooth muscle* | − | − | − | +++ | + | + | +++ | ++ | ++ | − |
| Platelets | − | − | − | ++ | +++ | ++ | − | ++ | − | + |
| Fibroblasts | − | − | − | ++ | +++ | +++ | − | ++ | ++ | ++ |
| Epithelial cells | − | − | − | +++ | ++ | ++ | − | + | + | ++ |
| Lymphoid cells | − | − | − | − | ++ | +++ | − | − | − | − |

TABLE II

*Tropomyosin characteristics*

This table summarizes available data for the nine *Tm* proteins. A minus sign indicates negative results; a plus-minus sign indicates that results varied with sample used or were quantitatively different from results obtained for the majority of the *Tm* proteins; a plus sign indicates consistently positive results regardless of sample used. ND means not determined.

| | Tm:1 | Tm:2 | Tm:3 | Tm:4 | Tm:5 | Tm:6 | Tm:7 | Tm:8 | Tm:9 |
|---|---|---|---|---|---|---|---|---|---|
| Other names[a] | aTm | bTm | Cytosk:8 | Cytosk:11 | Cytosk:13 | − | − | Cytosk:9 | Cytosk:10 |
| *Urea shifts up*[b,c] | + | + | + | + | ± | + | + | + | + |
| No Pro to Trp[c,d] | + | + | + | + | + | ND | + | + | + |
| In Tm preparations | + | + | + | + | ± | + | ± | + | + |
| In fibrils[a-c] | + | + | + | + | + | + | + | + | + |
| Reacts with anti-Tm | + | + | + | + | ± | + | ± | + | + |
| pI 4.1 | + | + | + | + | + | + | + | + | + |
| $M_r$ 29,000 to 36,000 | + | + | + | + | + | + | + | + | + |

[a] Ref. 11.
[b] Unpublished data.
[c] Ref. 12.
[d] Ref. 30.

appearance of the new charge variant spot (*e.g.* Tm:4.1) (11, 12). Tm:1 is found in both skeletal and cardiac muscle as a predominant protein, while Tm:1′ and Tm:2 are found only in skeletal muscle. Tm:3, one of the major smooth muscle tropomyosins, is also found in nonmuscle cells such as platelets, epithelial cells, and fibroblasts. The major nonmuscle tropomyosin, Tm:4, and the adjacent protein, Tm:5, are found in every nonmuscle cell line and tissue analyzed thus far. The small amount of Tm:4 and :5 found in uterus patterns (Fig. 1*C*) may indicate connective tissue contamination or may represent co-expression of smooth muscle and nonmuscle proteins. Tm:6 appears to be specific to smooth muscle (possibly to uterus). Tm:7 is found in small amounts in uterus, platelets, epithelial cells, and fibroblasts; Tm:8 is present in uterus, fibroblasts, and epithelial cells, Tm:9 occurs in platelets, fibroblasts, and epithelial cells.

## DISCUSSION

The present study demonstrated the existence of a family of proteins with characteristics of tropomyosin that are differentially expressed in various cell types. Four of the nine *Tm* proteins described correspond to well-known tropomyosins in animal and human systems: Tm:1 and :2 correspond to skeletal muscle $\alpha$- and $\beta$-tropomyosins, respectively; Tm:3 to smooth muscle tropomyosin; and Tm:4 to the 30,000 molecular weight nonmuscle tropomyosin. Our finding that cardiac tropomyosin is similar to $\alpha$-tropomyosin from skeletal muscle is in agreement with the report of identical amino acid sequences for the one tropomyosin protein found in human cardiac muscle and $\alpha$-tropomyosin from human skeletal muscle (30). Tm:1′ appears to be a molecular weight variant of Tm:1; the peptide cleavage products of the two proteins are almost identical and the two proteins vary in abundance according to the Type I/Type II fiber ratio of the muscle sampled.[2] The remaining five *Tm* proteins (Tm:5, :6, :7, :8, and :9) have not been previously investigated, although Garrels (8) described a nonmuscle tropomyosin in L6 myoblasts (termed nm′) that probably corresponds to Tm:8. Tm:5, :6, :7, and :9 are not resolved from other tropomyosin proteins by one-dimensional SDS-PAGE, and, therefore, have been overlooked in previous studies. Tm:4 and :5, and also Tm:3, :6, and :7, for example, have similar molecular weights and would appear as one protein band on SDS-PAGE. With the data available, previous investigators tried to explain the function of nonmuscle tropomyosin in terms of the protein interactions known to exist in the striated fibers of skeletal muscle. The discovery of multiple tropomyosin proteins within one cell type suggests that new models must be devised to explain multiple functions for these proteins or interaction of different tropomyosin proteins to serve similar functions. Skeletal muscle fibers simply contract to exert force or relax. The contractile requirements of nonmuscle cells, however, can be visualized as more complex. Nonmuscle cells must rearrange their organelles during mitosis, transport intracellular particles, reversibly produce cellular projections (*e.g.* pseudopods), and, in some cases, attach to surfaces. Such complex activities understandably require complex machinery and controls.

Tm:1, :2, :3, :4, :6, :8, and :9 satisfy many of the classical characteristics used to define tropomyosin (Table II). These characteristics include stability in organic solvents and in boiling water, precipitation at pH 4.1, shift to a higher apparent molecular weight in urea plus SDS rather than in SDS alone (11, 12, 25), and lack of proline and tryptophan (8, 11, 12, 31). In addition, all of these proteins react with antibody directed against skeletal muscle tropomyosin and are associ-

ated either with the cytoskeletons of nonmuscle cells (11, 12, 15) or the myofibrils of muscle tissue.[3]

Tm:5 and :7 also exhibit many characteristics of tropomyosin and are, therefore, included in the *Tm* protein set, although they are not reproducibly isolated along with the other *Tm* proteins by tropomyosin purification. Tm:5 was not isolated in platelet tropomyosin preparations or fibroblast tropomyosin preparations in which platelets or brain were used as nonradiolabeled carrier protein. Tm:5 was, however, found reproducibly in lymphoid cell tropomyosin preparations and fibroblast tropomyosin preparations with unlabeled lymphoid cells as carrier protein. Tm:5 lacks proline and tryptophan (11, 12) as is expected for a tropomyosin protein, but its apparent molecular weight does not shift as much as that of Tm:4 on urea/SDS electrophoresis (Cytosk:13 in Ref. 11, Fig. 2). The relative abundances of Tm:4 and :5 are different for lymphoid and nonlymphoid cells, with Tm:5 being more abundant than Tm:4 in lymphoid cells, but less abundant in platelets, fibroblasts, and epithelial cells (Fig. 1 and Ref. 15). Like Tm:5, Tm:7 was found in fibroblast tropomyosin preparations only when lymphoid cells were used as nonradioactive carrier. Tm:7 is also present in uterus and platelet tropomyosin preparations and, to a lesser extent, in epithelial cell whole homogenates. The anomalous characteristics of Tm:5 and Tm:7 relative to the other *Tm* proteins suggest that these proteins could be closely associated in some way with the tropomyosin protein(s), if they are not actual tropomyosin proteins. However, the majority of the properties of Tm:5 and :7 makes their identification as tropomyosins very likely.

Platelet tropomyosin has been previously described as having two components, $\alpha$ and $\beta$, both with molecular weights of approximately 30,000 (32). The two polypeptides are so similar in molecular weight that they appear as one band when one-dimensional electrophoresis is done according to Weber and Osborne (33) but are resolved into two bands with the discontinuous SDS-PAGE system described by Laemmli (34). The two-dimensional electrophoresis system used for our studies included the discontinuous SDS-PAGE as the second dimension separation, thus allowing separation of the two major platelet tropomyosins. Tm:4 corresponds to the $\alpha$-subunit, and Tm:5 most probably corresponds to the $\beta$-subunit. The major characterization studies done on platelet tropomyosin, including amino acid sequence analysis (4, 5, 35, 36), have concentrated on the $\alpha$-subunit and, therefore, refer to Tm:4 in this study. Our data suggest that three additional tropomyosin proteins (Tm:3, :7, and :9) are found in human platelets as well. The platelet preparations used for our studies ($10^6$ platelets/ml) contained no detectable erythrocytes or leukocytes; therefore, the appearance of Tm:3, :7, and :9 in platelet tropomyosin preparations must have been due to platelets alone. Platelet Tm:3, :7, and :9 have probably remained undescribed because they are such minor components relative to Tm:4 and :5 in the platelet preparations. As peptide cleavage demonstrated (Fig. 6), Tm:3 from platelets is identical to fibroblast Tm:3. In addition, antiserum to skeletal muscle tropomyosin that cross-reacts with uterine Tm:3 (12) also reacts with platelet Tm:3 on nitrocellulose transfers. Conversely, rabbit antiserum raised against platelet Tm:3 has been found to bind to uterine Tm:3 on nitrocellulose transfers.[3] These data indicate that Tm:3, :7, and :9 are tropomyosins that are normally found in platelets in addition to the previously reported nonmuscle tropomyosins, Tm:4 and :5.

Two-dimensional electrophoresis patterns of Tm:8 and :9 in fibroblasts and epithelial cells are similar to those of brain clathrin light chains described by Keen *et al.* (37). Clathrin light chains from bovine brain have molecular weights between 30,000 and 36,000 (37), are resistant to heat denaturation (38), have acidic isoelectric points (37), and cross-react with anti-tropomyosin antibodies (39). Unlike the tropomyosin-like proteins described in this study, however, clathrin light chains do not show an altered mobility in SDS-urea *versus* SDS alone (40), they are soluble in 0.5% NP40 (41) and thus are not associated primarily with the detergent-resistant cytoskeletons of cells, and they are precipitated predominantly by 30–40% ammonium sulfate rather than by 40–53% as is nonmuscle tropomyosin (37). Therefore, the present data indicate that none of the *Tm* proteins are likely to be clathrin light chains, although they may be structurally related in some way. Future studies on the reactivity of Tm:8 and :9 with monoclonal antibodies against clathrin light chains will provide further information on the relationship of these proteins.

The data indicate that each of the *Tm* proteins is the product of a different gene. Comparison of peptide fragments after partial proteolysis showed, with the exception of Tm:8, that each protein has a distinct primary structure because different cleavage patterns were obtained after digestion with V8 protease or chymotrypsin (Fig. 4). The cleavage patterns of Tm:1 and :8 were almost identical, but close examination revealed that after proteolysis with chymotrypsin, Tm:8 was either missing or had a much smaller amount of a peptide present in Tm:1 (*arrows* in Fig. 4*C*). In addition, Tm:1 and :8 did not co-migrate (Figs. 5 and 7). The observation that large amounts of Tm:1 pushed Tm:8 aside but did not dilute the radioactively labeled Tm:8 (Fig. 5) indicates that SDS complexes of the two proteins have distinct isotachophoretic properties, even though they have nearly identical pI values and molecular weights.

The independent charge variants described previously for Tm:3 and :4 from human skin fibroblasts (11, 12), together with our peptide cleavage data, indicate that those two tropomyosin-like proteins are coded for by genes distinct from one another and from those coding for the other fibroblast *Tm* proteins. DNA for Tm:3 and :4 is currently being isolated and sequenced by use of fibroblast lines that carry the variant proteins Tm:3.1 and Tm:4.1.[4] Co-expression of Tm:3 and :4 has been observed in numerous cell types including skin fibroblasts, epithelial cells, platelets, and erythrocytes.[3] A small amount of Tm:4 was also present in both whole homogenates and tropomyosin preparations from human myometrium. Caution must be exercised when interpreting the appearance of multiple forms of tropomyosin in tissue homogenates, however, because connective tissue or vascular contamination in the biopsy material used could contribute significant amounts of protein. Cells grown in culture offer a more homogeneous population, but they might synthesize smooth muscle tropomyosin (Tm:3) as a response to *in vitro* conditions just as all cultured cells synthesize vimentin (42) whether or not their *in vivo* counterparts do. The co-expression of both Tm:3 and Tm:4 in cells from the peripheral blood (platelets and erythrocytes), however, shows that cells can synthesize more than one tropomyosin *in vivo* and suggests that the synthesis of Tm:3 by cultured cells reflects some function(s) they share with platelets.

The reasons for co-expression of multiple tropomyosins by some cells and not by others remain to be elucidated. Lymphoid cells are unique among the cell types analyzed thus far in

[3] C. S. Giometti, unpublished observations.

[4] A. MacLeod, personal communication.

their limited expression of Tm:4 and :5, both tropomyosin-like proteins that are characteristic of nonmuscle cells. All other cell types analyzed, both from tissue culture and peripheral blood, express Tm:3 and :8, proteins also found in smooth muscle tissue. Such co-expression raises interesting questions. Do the smooth muscle tropomyosins function in nonmuscle cells, or is their expression extraneous? Do the different tropomyosins, muscle and nonmuscle, each function in different cellular processes (*e.g.* cell motility *versus* organelle motility)? Tm:1 and :2 are known to be subunits of skeletal muscle tropomyosin. Which of the remaining seven tropomyosin proteins are associated as native tropomyosin in smooth muscle and nonmuscle cells? Matsumura and coworkers (9, 10) have shown that the tropomyosin proteins in virally transformed rat cells differ quantitatively from normal controls. Are there similar quantitative variations in the different tropomyosins in human cancer cells and, if so, what significance do these variations have in the course of the disease? Elucidation of the interactions of these multiple tropomyosin species, the significance of the more specifically expressed and less abundant species (*e.g.* Tm:6 and :9), and the function of these proteins within the cell will require detailed analysis, both biochemical and immunochemical, of each individual protein. The results presented here are intended to provide as complete a catalog of the tropomyosin family as current protein mapping technology will allow and thereby to provide a basis for such comparative functional studies. Considered as a prototype for large-scale protein indexing efforts (43), the richness of the tropomyosin catalog suggests that similar comprehensive efforts will yield valuable insight into the functional specialization and the expression systematics of other important cellular proteins.

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REFERENCES

1. Fine, R. E., Blitz, A. L., Hitchcock, S. E., and Kaminer, B. (1973) *Nat. New Biol.* **245,** 182–186
2. Fine, R. E., and Blitz, A. L. (1975) *J. Mol. Biol.* **95,** 447–454
3. Bretscher, A., and Weber, K. (1978) *FEBS Lett.* **85,** 145–148
4. Cohen, I., and Cohen, C. (1972) *J. Mol. Biol.* **68,** 383–387
5. Côté, G. P., and Smillie, L. B. (1981) *J. Biol. Chem.* **256,** 11004–11010
6. Der Terrossian, E., Fuller, S. D., Stewart, M., and Weeds, A. G. (1981) *J. Mol. Biol.* **153,** 147–167
7. Schloss, J. A., and Goldman, R. D. (1980) *J. Cell Biol.* **87,** 633–642
8. Garrels, J. I. (1979) *Dev. Biol.* **73,** 134–152
9. Matsumura, F., Yamashiro-Matsumura, S., and Lin, J. J-C. (1983) *J. Biol. Chem.* **258,** 6636–6644
10. Matsumura, F., Lin, J. J-C., Yamashiro-Matsumura, S., Thomas, G. P., and Topp, W. C. (1983) *J. Biol. Chem.* **258,** 13954–13964
11. Giometti, C. S., and Anderson, N. L. (1981) *J. Biol. Chem.* **256,** 11840–11846
12. Giometti, C. S., and Anderson, N. L. (1984) *J. Mol. Biol.* **173,** 109–123
13. Talbot, K. K., and MacLeod, A. R. (1983) *J. Mol. Biol.* **164,** 159–174
14. Lin, J. J-C., Matsumura, F., and Yamashiro-Matsumura, S. (1984) *J. Cell Biol.* **98,** 116–127
15. Giometti, C. S., Willard, K. E., and Anderson, N. L. (1982) *Clin. Chem.* **28,** 955–961
16. Willard, K. E., and Anderson, N. G. (1981) *Clin. Chem.* **27,** 1327–1334
17. Anderson, N. G., and Anderson, N. L. (1978) *Anal. Biochem.* **85,** 331–340
18. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021
19. Anderson, N. L., and Anderson, N. G. (1978) *Anal. Biochem.* **85,** 341–354
20. Anderson, N. L., and Anderson, N. G. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 5421–5425
21. Bonner, W. M., and Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 83–88
22. Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) *J. Biol. Chem.* **252,** 1102–1106
23. Guevara, J., Jr., Johnston, D. A., Ramagali, L. S., Martin, B. A., Capetillo, S., and Rodriquez, L. V. (1982) *Electrophoresis* **3,** 197–205
24. Anderson, N. L., Nance, S. L., Pearson, T. W., and Anderson, N. G. (1982) *Electrophoresis* **3,** 135–142
25. Sender, P. M. (1971) *FEBS Lett.* **17,** 106–110
26. Anderson, N. L., and Hickman, B. J. (1979) *Anal. Biochem.* **93,** 312–320
27. Anderson, N. L., Taylor, J., Scandora, A. E., Coulter, B. P., and Anderson, N. G. (1981) *Clin. Chem.* **27,** 1807–1820
28. Anderson, N. L., Tracy, R., and Anderson, N. G. (1984) in *The Plasma Proteins* (Putnam, F., ed) Academic Press, New York, in press
29. Anderson, N. L., Giometti, C. S., Gemmell, M. A., and Macy, M. (1984) *Ann. N. Y. Acad. Sci.*, **428,** 134–143
30. Romero-Herrera, A., Nasser, S., and Lieska, N. (1982) *Muscle Nerve* **5,** 713–718
31. Mak, A. S., Smillie, L. B., and Stewart, G. R. (1980) *J. Biol. Chem.* **255,** 3647–3655
32. Côté, G., Lewis, W. G., and Smillie, L. B. (1978) *FEBS Lett.* **91,** 237–241
33. Weber, K., and Osborne, M. (1975) in *The Proteins* (Neurath, H., and Hill, R. L., eds) Vol. 1, pp. 180–225, Academic Press, New York
34. Laemmli, U. K. (1970) *Nature (Lond.)* **227,** 680–685
35. Côté, G. P., and Smillie, L. B. (1981) *J. Biol. Chem.* **256,** 7257–7261
36. Lewis, W. G., Cote, G. P., Mak, A. S., and Smillie, L. B. (1983) *FEBS Lett.* **156,** 269–273
37. Keen, J. H., Willingham, M. C., and Pastan, I. (1981) *J. Biol. Chem.* **256,** 2538–2544
38. Brodsky, F. M., Holmes, N. J., and Parham, P. (1983) *J. Cell Biol.* **96,** 911–914
39. Puszkin, P., Maimon, J., and Schook, W. (1979) *J. Cell Biol.* **83,** 293a
40. Ungewickell, E., and Branton, D. (1981) *Nature (Lond.)* **289,** 420–422
41. Brodsky, F. M., and Parham, P. (1983) *J. Mol. Biol.* **167,** 197–204
42. Franke, W. W., Schmid, E., Winter, S., Osborn, M., and Weber, K. (1979) *Exp. Cell Res.* **123,** 25–46
43. Anderson, N. G., and Anderson, L. (1982) *Clin. Chem.* **28,** 739–748