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An Electrophoretic Variant of a Human Fibroblast Protein with Characteristics of Smooth Muscle Tropomyosin

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A charge variant of a protein (Tm: 3; molecular weight approximately 35,000) that co-migrates with human smooth muscle tropomyosin has been found in whole cell extracts from the fibroblasts of a father and his son. The variant protein co-purifies with Tm: 3, shifts with it to a higher apparent molecular weight on sodium dodecyl sulfate/polyacrylamide gel electrophoresis in the presence of 4 M-urea, is a component of the microfilaments, and has a peptide cleavage pattern identical to that of Tm: 3 and smooth muscle tropomyosin. The results indicate that Tm: 3 and the variant (Tm: 3.1) are smooth muscle tropomyosin, suggesting that normal human fibroblasts synthesize at least two tropomyosins (Tm: 3 and the non-muscle tropomyosin Tm: 4, molecular weight approximately 30,000) and that they are the products of separate genes.

1. Introduction

Skeletal muscle tropomyosin, together with troponin, regulates the interaction of actin and myosin, thereby controlling muscle contraction and relaxation. Interspecies comparisons of skeletal muscle tropomyosins have consistently shown two subunits with molecular weights, in the presence of sodium dodecyl sulfate, of approximately 32,000 and 36,000 (Cummins & Perry, 1973). Cardiac muscle tropomyosin, in contrast, has only one subunit in mammals with small, fast-beating hearts (e.g. rabbits) but has two subunits in mammals with large, slow-beating hearts (e.g. humans) (Mak *et al.*, 1978). The amino acid sequence of the single subunit of rabbit cardiac muscle tropomyosin is identical to that of rabbit skeletal muscle α-tropomyosin (Lewis & Smillie, 1980). Smooth muscle, which is morphologically quite different from the striated skeletal and cardiac muscles, has been found to have either one or two tropomyosin subunits, depending on the tissue analyzed (Cummins & Perry, 1974). Although the fastest migrating smooth muscle tropomyosin moves to a position close to skeletal muscle β-tropomyosin, the smooth muscle proteins have no immunological identity with the skeletal muscle tropomyosin. In addition, the smooth muscle tropomyosins

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contain more histidine, glutamic acid and valine, but less lysine, isoleucine and tyrosine, than the skeletal muscle tropomyosins. The data suggest, therefore, that the muscle tropomyosins are coded for by at least three different genes, one each for the α- and β-tropomyosins of skeletal and cardiac muscle and a third gene for smooth muscle tropomyosin.

In addition to the tropomyosins isolated from different types of muscle, tropomyosin-like proteins have been isolated from non-muscle cells and tissues, including platelets (Cohen & Cohen, 1972; Coté & Smillie, 1981), brain (Fine *et al.*, 1973; Fine & Blitz, 1975), pancreas (Fine & Blitz, 1975) and fibroblasts (Fine & Blitz, 1975; Schloss & Goldman, 1980; Giometti & Anderson, 1981). These proteins are similar to the muscle tropomyosins in that they are stable at high temperatures and in organic solvents, are insoluble at pH 4·1, exhibit a slower migration on sodium dodecyl sulfate/polyacrylamide gel electrophoresis in the presence of urea than in its absence and contain little or no proline. Unlike the muscle tropomyosins, the non-muscle proteins all have molecular weights of approximately 30,000; furthermore, the axial repeat of their paracrystals is shorter than that of the muscle tropomyosins (Cohen & Cohen, 1972; Coté & Smillie, 1981; Fine *et al.*, 1973). The non-muscle tropomyosins, therefore, appear to represent a fourth type of tropomyosin coded for by yet another gene.

The different types of tropomyosin have appeared to be cell-specific, although a protein with a molecular weight of 35,000 to 36,000 was reported as a minor component of several non-muscle tropomyosin preparations (Fine & Blitz, 1975; Schloss & Goldman, 1980). This minor protein was assumed to be smooth muscle tropomyosin from contaminating vascular tissue in the starting material. In pure cultures of human fibroblasts, however, we have detected a protein that co-migrates with smooth muscle (human uterus) tropomyosin (approximate molecular weight 35,000) in addition to the characterized non-muscle tropomyosin (molecular weight 30,000). In addition, we have found a charge variant of this 35,000 molecular weight protein in two genetically related fibroblast lines. Biochemical characterization of this charge variant (named Tm: 3.1) and the normally expressed 35,000 molecular weight protein (named Tm: 3, where Tm: 1 is α-tropomyosin and Tm: 2 is β-tropomyosin from skeletal muscle) suggests that normal human fibroblasts synthesize both smooth muscle tropomyosin and non-muscle tropomyosin, and that the two proteins are the products of separate genes.

2. Materials and Methods

(a) *Cell cultures*

Cell line GM1386 from the American Type Culture Collection was used for the biochemical characterization of Tm: 3 and Tm: 3.1. Approximately 10^5 cells were grown for 24 h in flat-bottomed multiwell plates (Falcon 3047) with Eagle's MEM medium (GIBCO, Grand Island, New York) containing 10% fetal bovine serum and antibiotics (gentamycin, penicillin and streptomycin). The medium was then replaced with RPMI 1640 (GIBCO) minus methionine or with RPMI 1640 containing 25% of the normal concentration of total amino acids with 5% fetal bovine serum and antibiotics. Radiolabeled amino acids (50 μCi of [^{35}S]methionine/ml (1150 Ci/mmol), 2·5 μCi of [^{14}C]proline/ml (285 mCi/mmol) or 2·5 μCi of [^{14}C]tryptophan/ml (58 mCi/mmol)) were

added to each well, and the cultures were incubated for an additional 18 h before preparation of cytoskeletons or harvesting of cells.

(b) *Cytoskeleton preparation*

Fibroblast cytoskeletons were prepared by incubating the cells for 10 min at room temperature in buffer containing 0·1 M-piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), 0·001 M-$MgSO_4$, 2·0 M-glycerol, 0·5% (v/v) Nonidet P40 and 0·002 M-ethyleneglycol-bis(β-aminoethylether)-*N*,*N*′-tetraacetic acid (for preparation of complete cytoskeletons) or 0·001 M-$CaCl_2$ (for preparation of cytoskeletons without microtubules) (pH 6·9). Phenylmethylsulfonyl fluoride (1 mM) and pepstatin A (1 mM) were included to prevent proteolysis. Both microfilaments and microtubules were extracted from cytoskeletons using the low salt and high salt solutions described by Bravo *et al.* (1982) for the isolation of intermediate filaments. Cytoskeletons were harvested by washing each well with 50 μl of Nonidet P40–urea mixture (9 M-urea, 4% Nonidet P40, 2% (v/v) ampholytes (LKB, pH 9–11) and 2% (v/v) 2-mercaptoethanol).

(c) *Tropomyosin purification*

Smooth muscle (human uterus) and fibroblast tropomyosins were purified according to the method of Fine *et al.* (1973). Human platelets (1·5 g wet weight) were used as a non-radioactive protein carrier for the radiolabeled-fibroblast preparation.

(d) *Two-dimensional electrophoresis*

Isoelectric focusing was done as described by Anderson & Anderson (1978*a*). The second-dimension sodium dodecyl sulfate/polyacrylamide gel electrophoresis was done on linear 8% to 18% (w/v) polyacrylamide gels as described by Anderson & Anderson (1978*b*). For experiments requiring urea in the second-dimension separation, the slab gels were cast with acrylamide containing 4 M-urea. Electrophoresis, staining and destaining were all done as described (Anderson & Anderson, 1977). Dried gels were autoradiographed or fluorographed (Bonner & Laskey, 1974) using Kodak XAR-2 film.

(e) *Nitrocellulose transfer and staining*

Uterine and fibroblast proteins were transferred from 2-dimensional electrophoresis gels onto sheets of nitrocellulose (Schleicher and Schuell, Inc.) as described by Anderson *et al.* (1982). The nitrocellulose sheets were then stained with 0·1% Amido black in 45% ethanol/10% acetic acid or blocked with 3% bovine serum albumin (Miles Laboratories) in 10 mM-Tris–saline (pH 7·5). Blocked nitrocellulose transfers were incubated for 1 h at room temperature with rat anti-chicken leg muscle tropomyosin (a gift from Dr Alexander MacLeod, M.R.C. Centre, Cambridge, England; diluted 1 : 50 in bovine serum albumin/Tris–saline), followed by a 1-h incubation with peroxidase-conjugated rabbit anti-rat immunoglobulin G (Miles Laboratories; diluted 1 : 1000 in bovine serum albumin/Tris–saline). After several washes with saline, the spots with bound antibody were detected using horseradish peroxidase reagent containing 4-chloro-1-naphthol (BioRad Laboratories).

(f) *Peptide mapping by limited proteolysis*

Protein spots were cored from 2-dimensional electrophoresis gels and digested with trypsin (50 μg/ml) or *Staphylococcus aureus* V8 protease (50 μg/ml) as described (Giometti & Anderson, 1981; Cleveland *et al.*, 1977). Separation of the digestion products was done on 18% polyacrylamide slab gels. The final digestion products were detected using the silver stain described by Guevera *et al.* (1982).

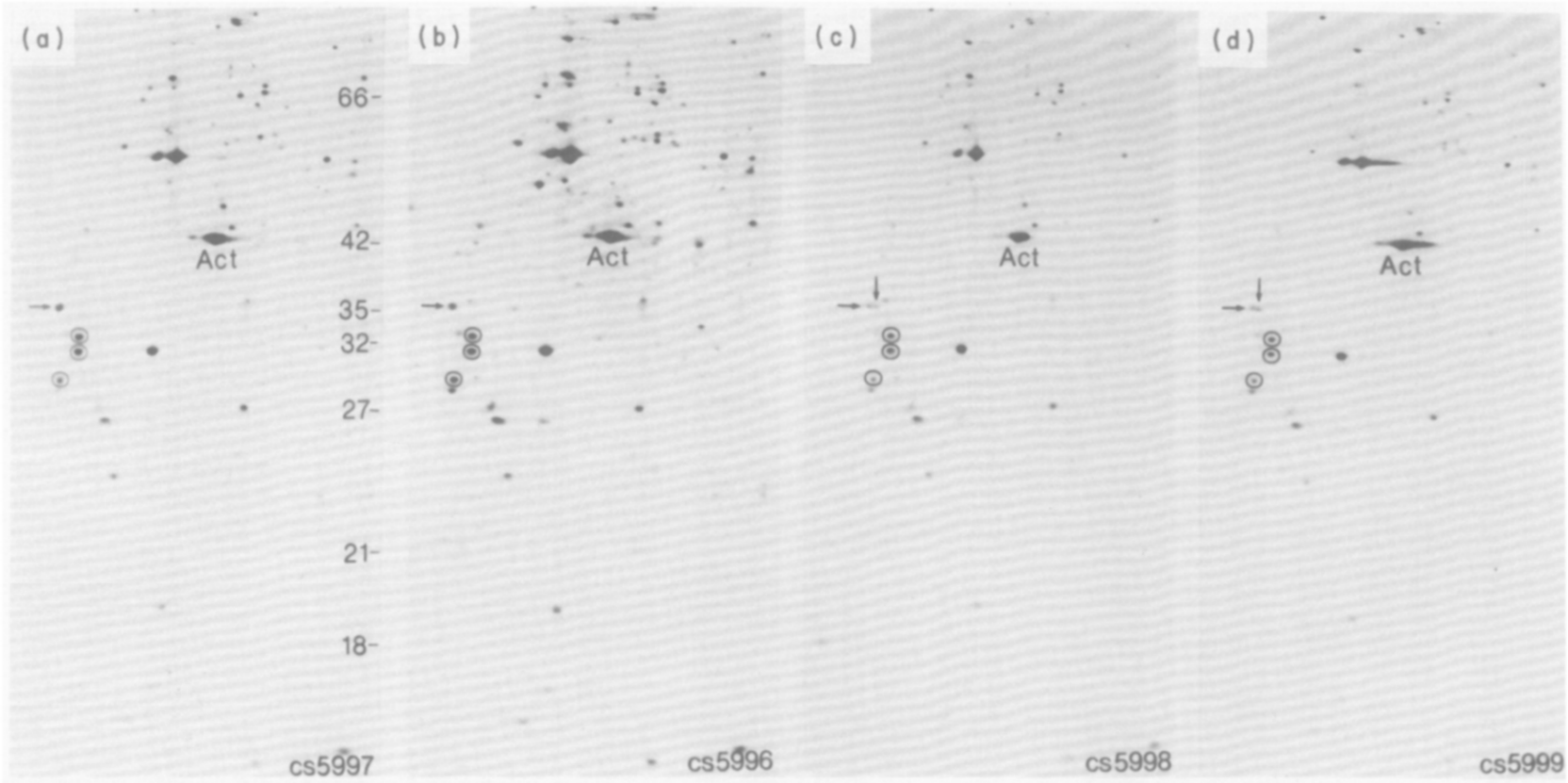

FIG. 1. Two-dimensional electrophoresis patterns of human skin fibroblast lines. Cells were labeled with [^{35}S]methionine and prepared for electrophoresis as described in Materials and Methods. Proteins were separated by 2-dimensional gel electrophoresis at Argonne National Laboratory as described in Materials and Methods. Patterns are oriented with the acidic side to the left and the basic side to the right. Act. indicates the position of actin. The horizontal arrows indicate the 35,000 molecular weight protein (Tm3; (Cytosk8)) common to all human skin fibroblasts; the vertical arrows indicate the protein variant (Tm3.1). Circles mark the protein spots used to calculate the scaling constant for Table 1. Approximate molecular weights ($\times 10^{-3}$) based on separation of rat heart proteins (Giometti *et al.*, 1980) are shown in (a). (a) GM1355 (female proband with hypercholesterolemia). (b) GM1354 (mother). (c) GM1385 (father) (d) GM1386 (son: brother of proband).

(g) *Nomenclature*

The complexity of 2-dimensional electrophoresis patterns necessitates a means of referring to specific proteins that have not yet been identified. In our laboratory we routinely name such proteins according to their membership in functionally or characteristically related protein sets, e.g. mitochondrial or cytoskeletal proteins (Anderson, 1981). These names, 6-letter acronyms followed by a serial number (e.g. Cytosk: 8), are not meant to replace the exact names of enzymes or structural proteins, once such identities have been determined. Thus, in this paper, we describe the identification of a fibroblast protein previously named Cytosk: 8 (Giometti & Anderson, 1981) as a smooth muscle tropomyosin. The abbreviation Tm: 3 has been chosen to refer to the major smooth muscle tropomyosin protein, with Tm: 1 and Tm: 2 referring to the α- and β-tropomyosins, respectively. Tm: 4 refers to the non-muscle tropomyosin previously called Cytosk: 11 (Giometti & Anderson, 1981). Other cardiac, smooth muscle and non-muscle proteins with tropomyosin-like characteristics have been given sequential numbers (Giometti & Anderson, 1982). Charge variants of these proteins have been assigned decimal designations in the order of their discovery, e.g. Tm: 3.1.

3. Results

Figure 1 shows a comparison of the two-dimensional electrophoresis patterns from four different lines of human skin fibroblasts. These four lines constitute a family set that was part of a genetic screen for protein markers of known metabolic disorders, in this case hypercholesterolemia. Although no differences in protein were found that correlated with the phenotypic expression of hypercholesterolemia, a charge variant was found in two of the four fibroblast lines. A protein with an approximate molecular weight of 35,000 appeared in all four lines, but an additional spot immediately to the right was found only in lines GM1385 (father) and GM1386 (son; brother of the proband with hypercholesterolemia). The more acidic (left) of these two spots is a cytoskeletal protein that was previously named Cytosk: 8 (see Materials and Methods, section (g)) and is found in fibroblast tropomyosin preparations (Giometti & Anderson, 1981). The more basic protein spot has not been seen in any of the other 100 fibroblast lines (animal and human) analyzed by this laboratory.

The variant protein and the protein immediately to the left were both associated with detergent-insoluble cytoskeletons prepared from GM1386 (Fig. 2(b)), and both proteins were also present in the cytoskeletons when microtubules were subtracted by disassembly in the presence of calcium chloride (Fig. 2(c)). When the microfilaments and microtubules were removed from the cytoskeletons by a cycle of low salt and high salt solutions, however, both proteins were missing from the two-dimensional electrophoresis pattern (Fig. 2(d)). These results illustrate the fact that both proteins are associated with the microfilaments.

Co-electrophoresis of fibroblast GM1386 labeled with [^{35}S]methionine and a sample of whole homogenate from human uterus (smooth muscle) showed that the fibroblast protein to the left of the variant protein co-migrated with a major smooth muscle protein thought to be smooth muscle tropomyosin (Tm: 3; Fig. 3(a) and (b)). Both muscle (Sender, 1971) and non-muscle (Giometti & Anderson, 1981; Garrels, 1979) tropomyosins have been shown to shift to a higher apparent

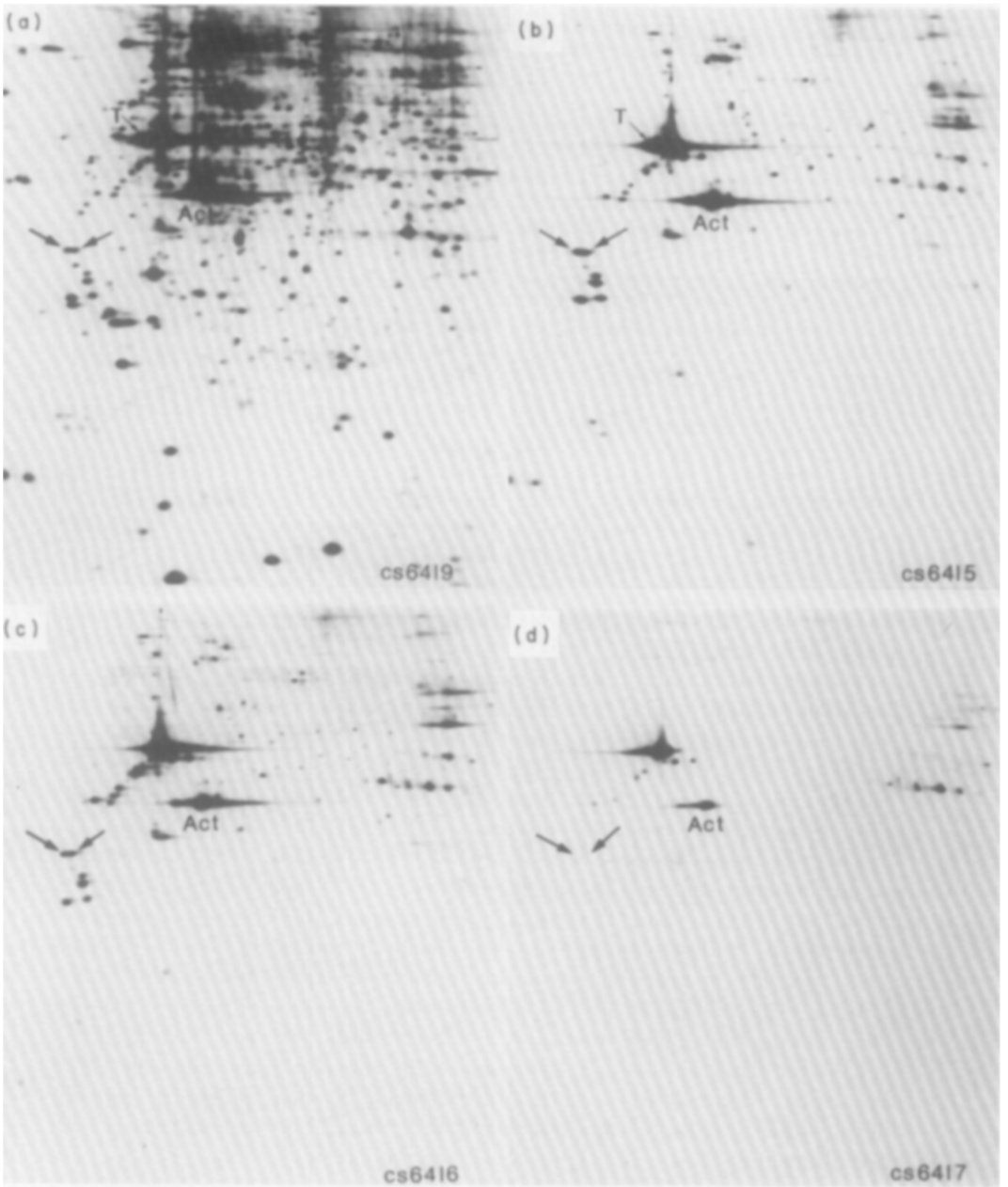

FIG. 2. Subcellular localization of protein variant Tm: 3.1. Cytoskeletons were prepared from GM1386 cells and proteins were separated as described in Materials and Methods. Patterns are oriented as in Fig. 1. Arrows indicate the position of Tm: 3 (left) and Tm: 3.1 (right). Act, actin; T, tubulin. (a) Whole cells. (b) Whole cytoskeletons (microtubules, microfilaments and intermediate filaments). (c) Cytoskeletons without microtubules. (d) Cytoskeletons without microtubules or microfilaments.

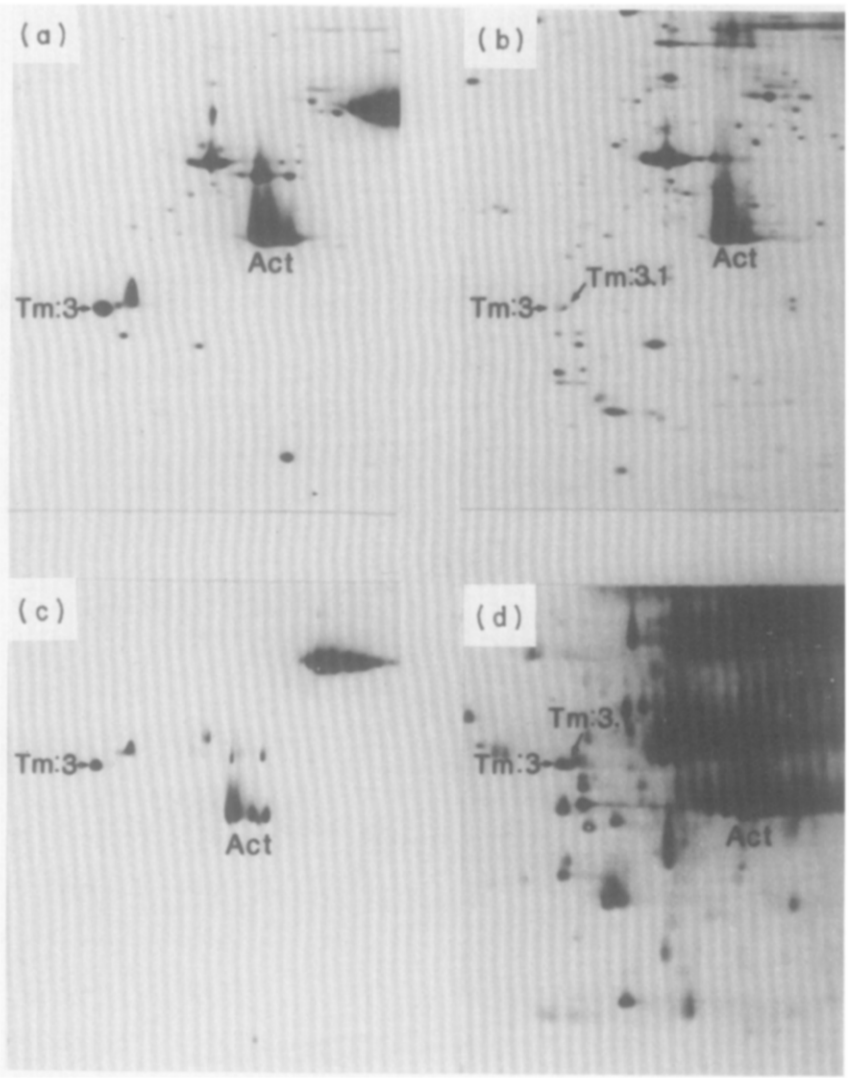

FIG. 3. Tm: 3 in smooth muscle and fibroblasts. (a) Coomassie blue pattern of human uterine homogenate and [^{35}S]methionine-labeled GM1386 cells with sodium dodecyl sulfate alone in the second-dimension separation. (b) Autoradiograph of the gel shown in (a). (c) Coomassie blue pattern of human uterine homogenate with sodium dodecyl sulfate and 4 M-urea in the second-dimension separation. (d) Autoradiograph of GM1386 proteins with sodium dodecyl sulfate and 4 M-urea in the second-dimension separation. Gel orientation and labels are as described for Fig. 1.

molecular weight when sodium dodecyl sulfate/gel electrophoresis is done in the presence of urea. When proteins from the smooth muscle homogenate and from GM1386 were separated in the second dimension with 4 M-urea, the smooth muscle Tm: 3 and fibroblast Tm: 3 and Tm: 3.1 (charge variant of Tm: 3) from GM1386 all migrated to a position above actin and had an apparent molecular

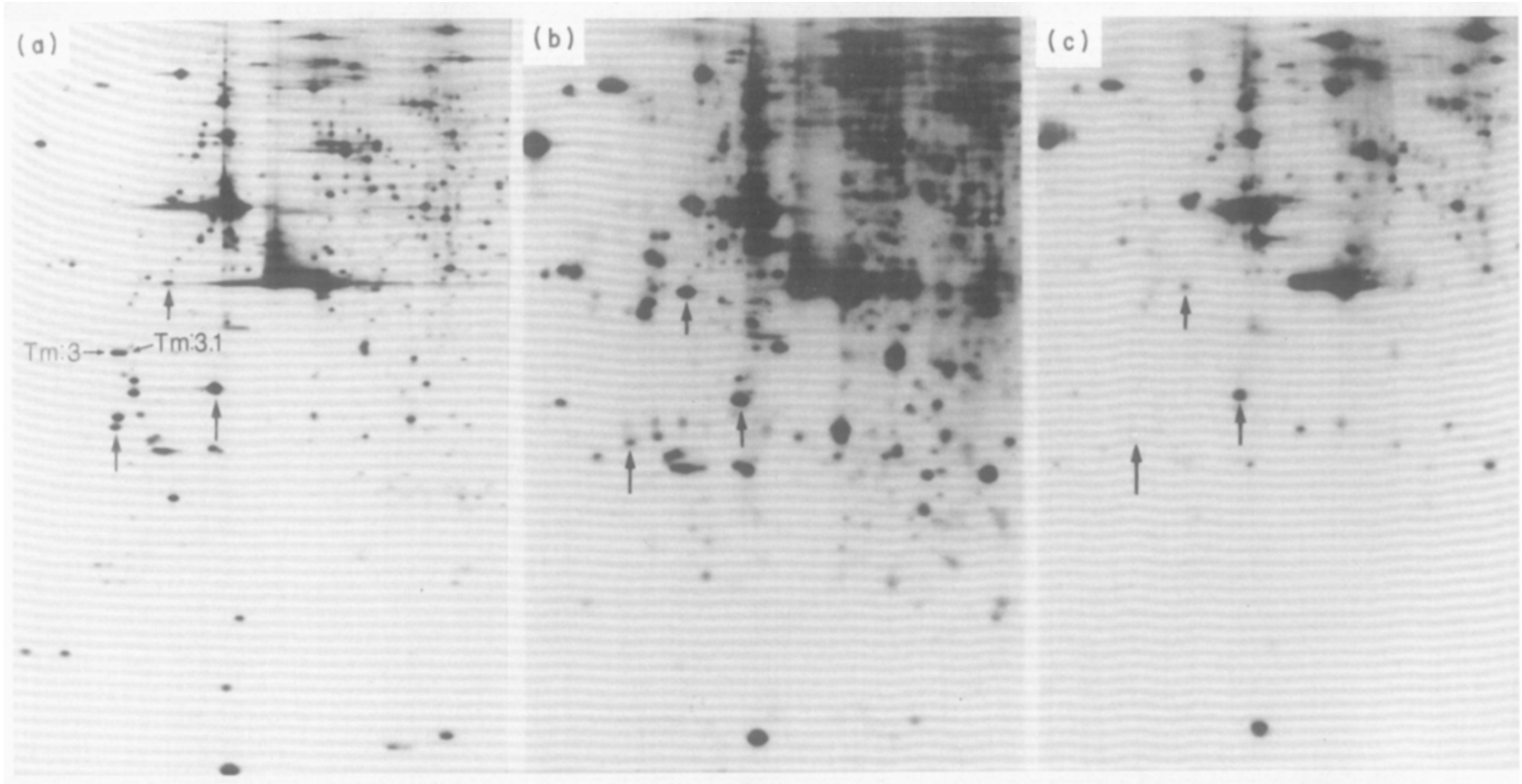

FIG. 4. Amino acid labeling of GM1386 cells with (a) [^{35}S]methionine, (b) [^{14}C]tryptophan or (c) [^{14}C]proline as described in Materials and Methods. The vertical arrows point to proteins that are present in all 3 samples and thus serve as markers. Gel orientation and labels are as described for Fig. 1.

weight of approximately 50,000 (Fig. 3(c) and (d)), compared to approximately 35,000 in the absence of urea.

Neither the muscle nor non-muscle tropomyosins for which the amino acid compositions are known contain proline or tryptophan (Cohen & Cohen, 1972; Fine *et al.*, 1973; Garrels, 1979; Mak *et al.*, 1980). When GM1386 was labeled with [^{14}C]proline or [^{14}C]tryptophan and the protein patterns were compared with the pattern obtained from cells labeled with [^{35}S]methionine, the protein that co-migrated with smooth muscle tropomyosin (Tm: 3) and the variant protein (Tm: 3.1) were both missing in the patterns of proline- and tryptophan-labeled cells (Fig. 4).

To characterize Tm: 3 further as smooth muscle tropomyosin, the procedure of Fine *et al.* (1973) was used to extract tropomyosin from a sample of human uterus. Two-dimensional electrophoresis of the uterine tropomyosin preparation resulted in the pattern shown in Figure 5(a). Although several proteins were present,

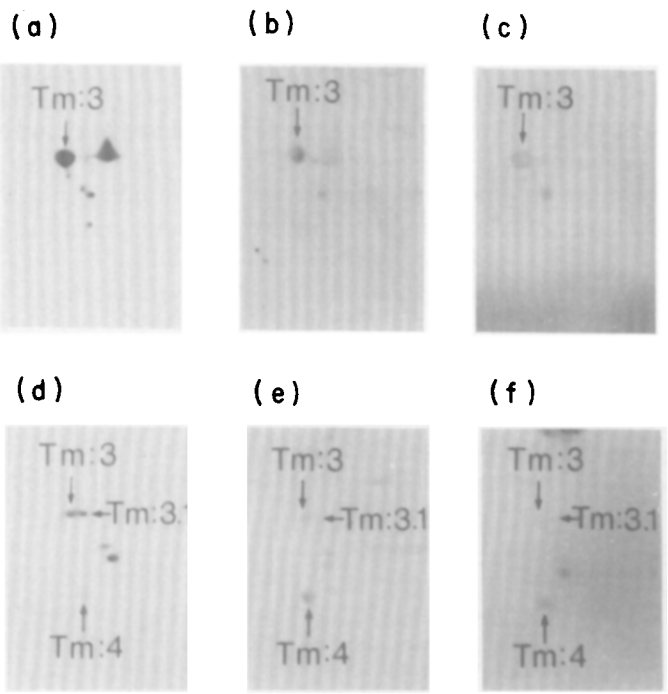

FIG. 5. Tropomyosin from human uterus and human skin fibroblasts (GM1386). (a) Coomassie blue-stained proteins of a tropomyosin preparation from 1·0 g (wet weight) of human uterus. (b) Nitrocellulose transfer of the tropomyosin region from a 2-dimensional gel pattern of human uterine proteins stained with Amido black. (c) Same as (b), except that the transfer was incubated with rat anti-chicken leg muscle tropomyosin as described in Materials and Methods for the detection of tropomyosin. (d) Autoradiograph of proteins from a tropomyosin preparation from [^{35}S]methionine-labeled GM1386 mixed with 1·0 g (wet weight) of human platelets as a cold carrier. Uterus was not used as a non-radioactive carrier in order to avoid the distortion of Tm: 3 and Tm: 3.1 that would occur due to a high concentration of uterine Tm: 3 (as seen in Fig. 3(b)). Tm: 4 (non-muscle tropomyosin) is slightly distorted due to the high concentration of carrier platelet protein that co-migrates. (e) Nitrocellulose transfer of the tropomyosin region from a 2-dimensional gel pattern of GM1386 fibroblast proteins stained with Amido black. (f) Same as (e), except that the transfer was reacted with rat anti-chicken leg muscle tropomyosin.

Tm: 3 was the most abundant protein in the preparation (as determined by spot size and staining intensity with Coomassie blue). Comparison of the two-dimensional electrophoresis pattern of purified uterine tropomyosin with a nitrocellulose transfer of uterine whole homogenate proteins stained with Amido black (Fig. 5(b)) showed that all but one of the minor proteins in the tropomyosin preparation were missing from the whole homogenate. This suggests that the remaining minor proteins were degradation products of higher molecular weight proteins resulting from the procedures used for tropomyosin purification. Staining of a nitrocellulose transfer of uterine whole homogenate proteins by reaction with rat antiserum against chicken leg muscle tropomyosin revealed Tm: 3 and one of the minor proteins present in the uterine tropomyosin preparation (Fig. 5 (c)). A protein with a molecular weight similar to Tm: 3, but slightly more basic, which was in the tropomyosin preparation, also showed a slight reaction with the rat anti-chicken tropomyosin. These results suggest that Tm: 3 is the major smooth muscle tropomyosin found in human uterus, although there are at least two other chemically distinct proteins with tropomyosin characteristics also present in the same tissue.

The two-dimensional electrophoresis pattern of a tropomyosin preparation from GM1386 fibroblasts is shown in Figure 5(d). This pattern is similar to one obtained previously, using a different fibroblast line (Giometti & Anderson, 1981), with the addition of the variant protein Tm: 3.1. A nitrocellulose transfer of GM1386 whole homogenate proteins stained with Amido black (Fig. 5(e)) showed that all of the major proteins found in the tropomyosin preparation were also present in the whole homogenate. A similar nitrocellulose transfer stained by reaction with rat anti-chicken leg muscle tropomyosin showed that all of the proteins seen in the tropomyosin preparation reacted with the antiserum. The two proteins with molecular weights intermediate between the weights of Tm: 3 and Tm: 4 that were found in the tropomyosin preparation and reacted with the anti-tropomyosin have also been found to have other tropomyosin-like characteristics that have been described elsewhere (Giometti & Anderson, 1982). Although some non-specific binding of the rat anti-chicken muscle tropomyosin to actin, vimentin and a 43,000 molecular weight fibroblast protein, which was more acidic than actin was observed (data not shown), the specificity of the antiserum was indicated by its failure to bind to the fibroblast protein next to Tm: 4 (similar molecular weight but more basic) and other minor proteins detected in the Amido black-stained patterns of both uterine and fibroblast proteins. These results indicate that Tm: 3 and Tm: 3.1 in GM1386 are related to the major tropomyosin protein isolated from human uterus tissue.

To confirm the identity of the variant protein as a charge variant of the Tm: 3 protein, both the Tm: 3 and Tm: 3.1 protein spots were cut from two-dimensional gels of GM1386 and digested with *S. aureus* V8 protease (peptide bond cleavage at the carboxyl end of aspartic and glutamic acid residues) or trypsin (peptide bond cleavage at the carboxyl end of lysine and arginine residues). The Tm: 3 protein spot from a two-dimensional electrophoresis gel of uterus homogenate and the spots known to be the α- and β-subunits of skeletal muscle tropomyosin were analyzed for comparison. Figure 6 shows that separation of the products of this

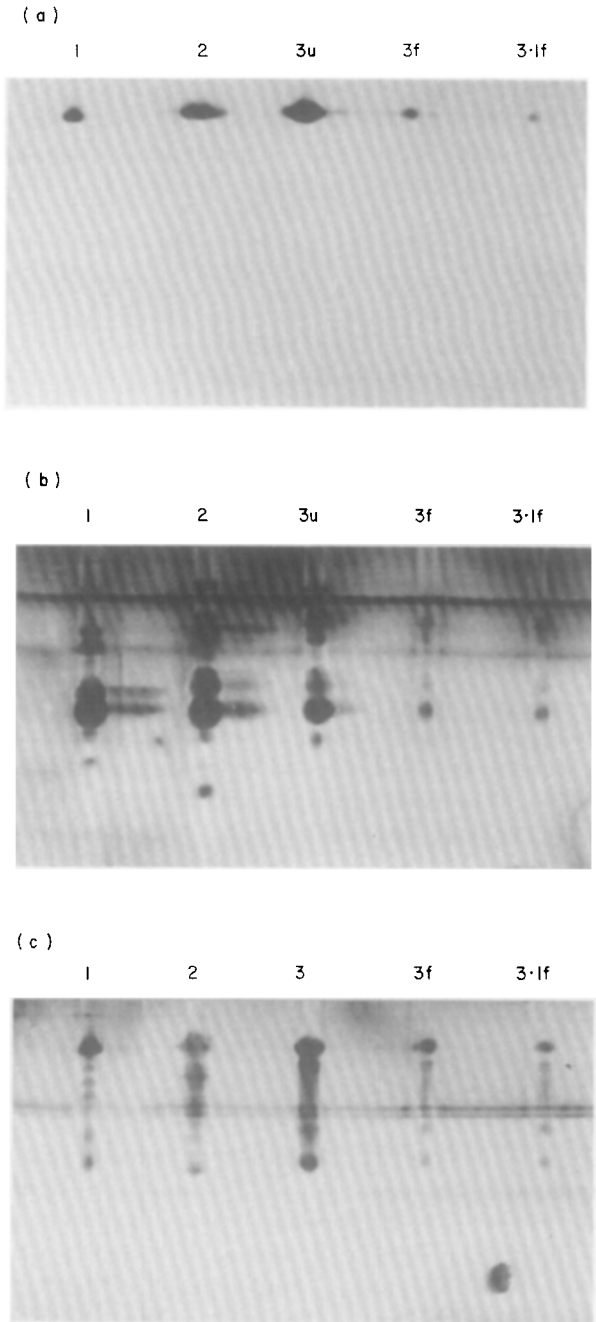

FIG. 6. Partial proteolysis of tropomyosin-like proteins from human skeletal muscle, uterus (smooth muscle) and GM1386 cells. Partial proteolysis of Coomassie blue-stained protein spots from 2-dimensional electrophoresis gels was done as described in Materials and Methods. Peptides were detected using the silver stain technique described by Guevera *et al.* (1982). The dark bands that stretch across the patterns in (b) and (c) are the enzymes used for proteolysis. (a) Control without protease treatment. (b) Products of *S. aureus* V8 protease digestion. (c) Products of trypsin digestion. 1, α-tropomyosin from skeletal muscle (Tm: 1); 2, β-tropomyosin from skeletal muscle (Tm: 2); 3u, Tm: 3 from uterus; 3f, Tm: 3 from GM1386 fibroblasts; 3.1f, Tm: 3.1 from GM1386 fibroblasts.

partial proteolysis by sodium dodecyl sulfate/gel electrophoresis produced identical banding patterns for Tm: 3 from uterus and GM1386 and for Tm: 3.1 from GM1386 when either *S. aureus* V8 protease or trypsin was used. The skeletal muscle tropomyosins each produced different cleavage patterns, both of which were distinct from the banding patterns obtained with Tm: 3 and Tm: 3.1.

4. Discussion

Protein heterogeneity has been reported in non-muscle tropomyosin preparations from brain (Fine & Blitz, 1975), baby hamster kidney cells, rat embryos and 3T3 cells (Schloss & Goldman, 1980). In each of these preparations, the major protein isolated had a molecular weight of approximately 30,000, while the minor protein component of the preparations had a molecular weight of approximately 35,000 to 36,000. Fine & Blitz (1975) suggested that the higher molecular weight protein in their brain tropomyosin preparation was derived from vascular smooth muscle that was in the brain tissue used as starting material. However, Schloss & Goldman (1980) found similar high molecular weight proteins in their tropomyosin preparations from pure cultures of both baby hamster kidney and 3T3 cells. Garrels (1979) has also described the existence of multiple forms of tropomyosin in prefusion myoblasts, one of which was described as smooth muscle tropomyosin. These last two reports suggest that the occurrence of more than one protein in tropomyosin preparations from non-muscle cells results from the co-expression of more than one tropomyosin gene in these cells.

We have previously described a charge variant of non-muscle tropomyosin (molecular weight 30,000; Tm: 3 (Cytosk: 11)) in a human skin fibroblast cell line (Giometti & Anderson, 1981). The tropomyosin preparations for that study contained three other proteins (molecular weights approximately 31,000 (Cytosk: 9), 32,000 (Cytosk: 10) and 35,000 (Cytosk: 8)), in addition to the 30,000 molecular weight protein. All three proteins were associated with the cytoskeleton and shifted to a higher apparent molecular weight in the presence of urea, just as the non-muscle tropomyosin did, but these proteins were labeled by [^{3}H]proline and therefore were not considered to be tropomyosin-like. More recent experiments, in which [^{14}C]proline was used instead of [^{3}H]proline, have revealed, however, that these proteins do not contain proline. The non-muscle tropomyosin in fibroblasts, previously reported to contain low amounts of proline based on the [^{3}H]proline experiments, was also found to contain no proline when the ^{14}C-labeled amino acid was used. Therefore, the ^{3}H incorporation in previous experiments must have been due to proton transfer leading to ^{3}H labeling of amino acids other than proline, and all four of the fibroblast proteins found in tropomyosin preparations lack proline. The occurrence of multiple tropomyosin-like proteins in fibroblasts has also been reported by Matsumura *et al.* (1982) and Talbot & MacLeod (1983).

The biochemical characterization of the 35,000 molecular weight fibroblast protein (Tm: 3) as smooth muscle tropomyosin was pursued after the discovery of an apparent genetically determined charge variant of that protein. Unlike the study in which a charge variant of non-muscle tropomyosin was described

TABLE 1

Integrated densities of spots Tm: 3 and Tm: 3.1

Cell line	Integrated density $\times 10^{-2}$ Tm: 3	Tm: 3.1
GM1354	17·4	
GM1355	21·7	
GM1385	8·81	5·19
GM1386	10·1	9·13

The autoradiograms shown in Fig. 1 were analyzed using the TYCHO system (Anderson *et al.*, 1981). Results were scaled so that the sum of the integrated densities of the spots circled in Fig. 1 was constant.

(Giometti & Anderson, 1981), a family set was available for this analysis. The variant protein (Tm: 3.1) was expressed by fibroblasts from the father and son, but not the mother or a daughter (proband). The appearance of the variant does not correlate with sex, since Tm: 3.1 was not found in any of the other 82 human fibroblast lines of either sex examined in this study. Tm: 3 and Tm: 3.1 are present in approximately equal abundance in GM1385 and GM1386, with Tm: 3 decreased to about half the amount found in GM1354 and GM1355, the lines without Tm: 3.1 (Table 1). These results suggest that GM1385 and GM1386 are heterozygotes in which one of the two genes that normally codes for Tm: 3 codes instead for Tm: 3.1. Fibroblast Tm: 3 co-migrated with the major tropomyosin protein of human uterus (defined by molecular weight shift in the presence of 4 M-urea during sodium dodecyl sulfate/gel electrophoresis, enrichment in tropomyosin preparations and reaction with rat anti-chicken leg muscle tropomyosin) and after partial proteolysis with *S. aureus* V8 protease or trypsin had a cleavage pattern identical to that of the corresponding protein from a whole homogenate of human uterus. These findings (together with the absence of proline and tryptophan, localization in the microfilaments, shift to a higher apparent molecular weight in the presence of urea, heat stability, stability in organic solvents and precipitation at pH 4·1) suggest not only that Tm: 3 is a tropomyosin, but also that it is a smooth muscle tropomyosin. Tm: 3.1 co-purifies with Tm: 3 in non-muscle tropomyosin preparations, is also associated with fibroblast microfilaments, lacks proline and tryptophan, shifts with Tm: 3 to a higher apparent molecular weight in the presence of urea, reacts with rat antiserum against chicken leg muscle tropomyosin and has a cleavage pattern identical to that of Tm: 3 after partial proteolysis. The conclusion may be drawn, therefore, that Tm: 3.1 is a charge variant of Tm: 3. Further verification of Tm: 3 as a smooth muscle tropomyosin could be obtained from analysis of smooth muscle tissue from the original donor of GM1386, since the variant protein Tm: 3.1 should also be present in the smooth muscle itself. Tm: 3.1 has no obvious effect on cell viability, growth or morphology. A similar lack of detrimental effects was found in the fibroblasts with a variant of non-muscle tropomyosin (Tm: 4).

The factors surrounding the co-expression of two different tropomyosin proteins in human fibroblasts remain to be explained. The occurrence of independent

charge variants of Tm: 3 and Tm: 4 demonstrates that the two proteins are the products of two distinct genes. It is unlikely that the expression of smooth muscle tropomyosin by human fibroblasts is caused by culture conditions, since the co-expression of Tm: 3 and Tm: 4 has also been seen in human platelets isolated from fresh samples of peripheral blood (unpublished results). It is interesting to note that the expression of α- and β-tropomyosins appears to be restricted to skeletal and cardiac muscle, both muscle systems that require tropomyosin and troponin for thin filament regulation of myosin–actin interactions (Coté & Smillie, 1981). Smooth muscle contraction, on the other hand, appears to be regulated by myosin-linked processes that can function independently of tropomyosin (Sobieszek & Small, 1977). Perhaps, the function of tropomyosin in smooth muscle, as yet undefined, is also required in certain types of non-muscle cells, thereby requiring the expression of the smooth muscle tropomyosin gene as well as the non-muscle tropomyosin gene. Immunochemical studies are in progress to determine the subcellular localization of Tm: 3 and Tm: 4 and to investigate the relationship of the two proteins within the intact cell.

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