THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 256, No. 22, Issue of November 25, pp. 11840–11846, 1981
Printed in U.S.A.

A Variant of Human Nonmuscle Tropomyosin Found in Fibroblasts by Using Two-dimensional Electrophoresis*

(Received for publication, January 28, 1981)

Carol S. Giometti and N. Leigh Anderson

From the Molecular Anatomy Program, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439

In an analysis of 12 human fibroblast cell lines by two-dimensional electrophoresis, one cell line (1493) was found to contain a major protein variant (Cytosk: 12; for the convention used in spot numbering, see Anderson, N. L. (1981) *Proc. Natl. Acad. Sci. U. S. A.* 78, 2407–2411) not present in any of the other 11 cell lines. Biochemical characterization of the variant protein included determination of its subcellular location, partial amino acid composition, behavior on sodium dodecyl sulfate (SDS) *versus* SDS/urea gels, and partial proteolytic digestion patterns. All of these methods showed that Cytosk:12 is related to Cytosk:11. Both proteins are located in the cytoskeleton, contain little cysteine or proline and no detectable tryptophan, shift together to a higher apparent molecular weight when electrophoresed in the presence of SDS and 8 M urea *versus* SDS alone, and have identical products after partial proteolysis according to Cleveland *et al.* (Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) *J. Biol. Chem.* 252, 1102–1106). Preparation of nonmuscle tropomyosin resulted in the partial purification of both Cytosk:11 and :12. The data suggest that Cytosk:11 is fibroblast nonmuscle tropomyosin and that Cytosk:12 in cell line 1493 is a charge variant of that protein.

Tropomyosin is a major protein of muscle that interacts with actin in the thin filaments. Analogous molecules appear to exist in many other cell types, suggesting that this protein may be ubiquitous. Nonmuscle tropomyosin has been isolated from human platelets (1), chick embryo brain (2), and mouse fibroblasts (3). In each case, the protein has shared such chemical characteristics with muscle tropomyosin as resistance to organic solvents and heat, precipitation at pH 4.1, and high α-helical content. The paracrystals formed from nonmuscle tropomyosins have an axial periodicity of 345 Å in contrast to the periodicity of 400 Å characteristic of muscle tropomyosins (1, 2). However, the approximate molecular weight of all nonmuscle tropomyosins, as judged by sodium dodecyl sulfate electrophoresis, described in the literature is only 30,000, compared to the SDS[1] molecular weights of 32,000 and 36,000 for the α and β forms of muscle tropomyosin. The ratio of the axial period of the tropomyosin paracrystals to the protein molecular weight is therefore comparable for muscle and nonmuscle tropomyosins, indicating analogy of structure. Chemical characterization of muscle and nonmuscle tropomyosins has clearly suggested that, although they are structurally and functionally similar, there are two distinct classes of tropomyosin protein. Expression of muscle and nonmuscle tropomyosins appears to be controlled by different genes with the higher molecular weight form being restricted to differentiated muscle cells (3).

Muscle tropomyosin is a heterogeneous protein. Two major forms are designated α and β, but additional heterogeneity within each form has been reported (4). The two major forms have substantially different SDS molecular weights and, although each has the same number of amino acid residues, the sequences differ at 39 positions (5). The ratio of α to β tropomyosin varies with the type of muscle, probably reflecting a difference in the function of the two forms. The α:β ratio is greater than 1 in muscle containing predominantly Type II fibers, but is almost equal to 1 in the muscles composed primarily of Type I fibers (6). At the present time, the physiological significance of the muscle tropomyosin heterogeneity is still not understood.

No analogous heterogeneity has been found among the nonmuscle tropomyosins. Similar molecular weights have been reported for all of the preparations studied, and there have been, to our knowledge, no studies using isoelectric focusing. Two-dimensional electrophoresis provides the opportunity to examine cellular proteins for both charge and molecular weight heterogeneity (7–9). During a two-dimensional electrophoretic analysis of human fibroblast cell lines, we have found a cytoskeletal protein that is present in only one of 12 cell lines studied with a molecular weight that is similar to that described for nonmuscle tropomyosins. In this paper, we describe the biochemical characterization of this variant protein and show that it is a charge variant of fibroblast tropomyosin. In cell line 1493, the variant protein is expressed in addition to the normal fibroblast tropomyosin protein. Computerized quantitation of the two-dimensional gel patterns suggests that the diploid cell normally contains two genes for nonmuscle tropomyosin, one of which is altered in the cell line 1493, thus producing the variant protein.

MATERIALS AND METHODS

Cell Cultures—Normal human skin fibroblasts (Table I) were received as a gift from Meloy Laboratories (Springfield, VA; National Cancer Institute Contract N01-CP91000). The experiments in this report were conducted with cells in passages 5–16. Cells were cultured in basal medium (Eagle's) with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (Grand Island Biological Co.), 10% fetal bovine serum, and 1% gentamycin. Cultures were started with 5×10^4 cells/well in flat-bottomed, multiwell plates (Falcon) and were grown in a humidified incubator (95% humidity with 5% CO_2) for 48 h. The semiconfluent cultures were then radiolabeled as described below.

Cell Radiolabeling—Fibroblast proteins were labeled by incubation for 16–18 h in RPMI 1640 medium minus specific amino acids

* This work was supported by Contract W-31-109-ENG-38 from the United States Department of Energy. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] The abbreviations used are: SDS, sodium dodecyl sulfate; NP-40, Nonidet P-40.

(Grand Island Biological Co.), supplemented with 5% fetal bovine serum, 2% glutamine, and one of the following: [^{35}S]methionine (100 μCi/ml of medium; Amersham/Searle), [^{3}H]histidine, [^{3}H]phenylalanine, [^{3}H]proline, or [^{3}H]tryptophan (all 40 μCi/ml of medium; Amersham/Searle). ^{14}C labeling of cells was performed by incubating the cells in 200 μl of phosphate-buffered saline containing 8 μCi of [^{14}C] iodoacetamide (Amersham/Searle) for 30 min at 25 °C. Labeled cells and cytoskeleton preparations (see below) were harvested for electrophoresis using 30 μl/well of a solution containing 9 M urea, 4% (v/v) Nonidet P-40, 2% (w/v) ampholytes (LKB; pH range 3.5–10), 5% (v/v) β-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride (subsequently referred to as NP-40/urea mix).

Cytoskeleton Preparation—Fibroblast cytoskeletons were prepared essentially as described by Osborn and Weber (10). Labeled cells were washed once with phosphate-buffered saline and then treated with 0.5% NP-40 and 1 mM phenylmethylsulfonyl fluoride in phosphate-buffered saline. After incubation for 10 min at 25°C, the detergent solution was removed, and the adhering cytoskeltons were washed once with phosphate-buffered saline.

Tropomyosin Purification—Fibroblasts were grown to semiconfluent layers in 75-cm^2 flasks and labeled with [^{35}S]methionine as described above. The labeled cells were scraped from each flask into 10 ml of absolute ethanol by using a rubber policeman. The resulting suspension was added to minced mouse brain (used as carrier protein) and was homogenized in a Waring blender. Tropomyosin was isolated according to the procedure outlined by Fine *et al.* (2) through the 53% ammonium sulfate step. The final precipitate was dissolved in a small amount of distilled water, dialyzed against distilled water, and lyophilized. The residue was mixed with 30 μl of NP-40/urea mix and analyzed by two-dimensional electrophoresis.

Two-dimensional Electrophoresis—The first dimension of isoelectric focusing was performed using the ISO apparatus as described previously (11) and LKB ampholytes (10% pH range 2.5–4, 90% pH range 3.5–10). The proteins were focused for 13,500 V-h at room temperature. Unless otherwise indicated, the second dimension of separation was performed on linear 10–20% acrylamide gradient gels as described previously (12). For experiments requiring urea in the second dimension gels, 10–20% acrylamide gradient gels containing 8 M urea were used. The gel pouring, running, staining, and destaining were performed as previously described (8). Gels containing proteins labeled with [^{35}S]methionine were autoradiographed (13), and those with proteins labeled with ^{3}H or ^{14}C were fluorographed (14). Kodak XR-5 film was used.

Peptide Mapping by Limited Proteolysis—Protein spots observed on autoradiographs were analyzed for relatedness by partial proteolytic digestion of the corresponding spot from the dried gel. The spots of interest were bored out of dried gels, placed into 500 μl of buffer (0.125 M Tris, pH 6.8, 0.1% SDS, and 1 mM EDTA), and incubated at 37 °C for 5 min. The rehydrated gel pieces were then placed at the top of a 3-cm deep, 3% acrylamide stacking gel lying over an 18% acrylamide separating slab gel. The stacking and separating gel compositions were as described by O'Farrell (7) with the addition of 0.1 mM EDTA. A cylinder of 4% acrylamide gel (1.5 mm diameter × 100 mm long) containing 50 μg/ml of chymotrypsin or trypsin in 0.125 M Tris, pH 6.8, 0.1% SDS, 0.1 mM EDTA was then put on top of the gel pieces and sealed into place with 0.5% agarose in 0.125 M Tris, pH 6.8, containing 0.1% SDS and 1 mM EDTA. The separation of the digestion products was accomplished as described by Cleveland *et al.* (15)

TABLE I

Human skin fibroblast lines analyzed by two-dimensional electrophoresis

Cell line	Sex	Age	Race
1239	F	46	C[a]
1242[b]	F	18	C
1243[b]	F	18	C
1291	M	17	C
1448	M	69	C
1493[c]	M	9	B[a]
1494[c]	F	9	B
1553	F	26	C
1559	F	52	C
1875	M	52	B
2685[b]	M	7	C
2686[b]	M	7	C

[a] C, Caucasian; B, black.
[b] Monozygous twins.
[c] Dizygous twins.

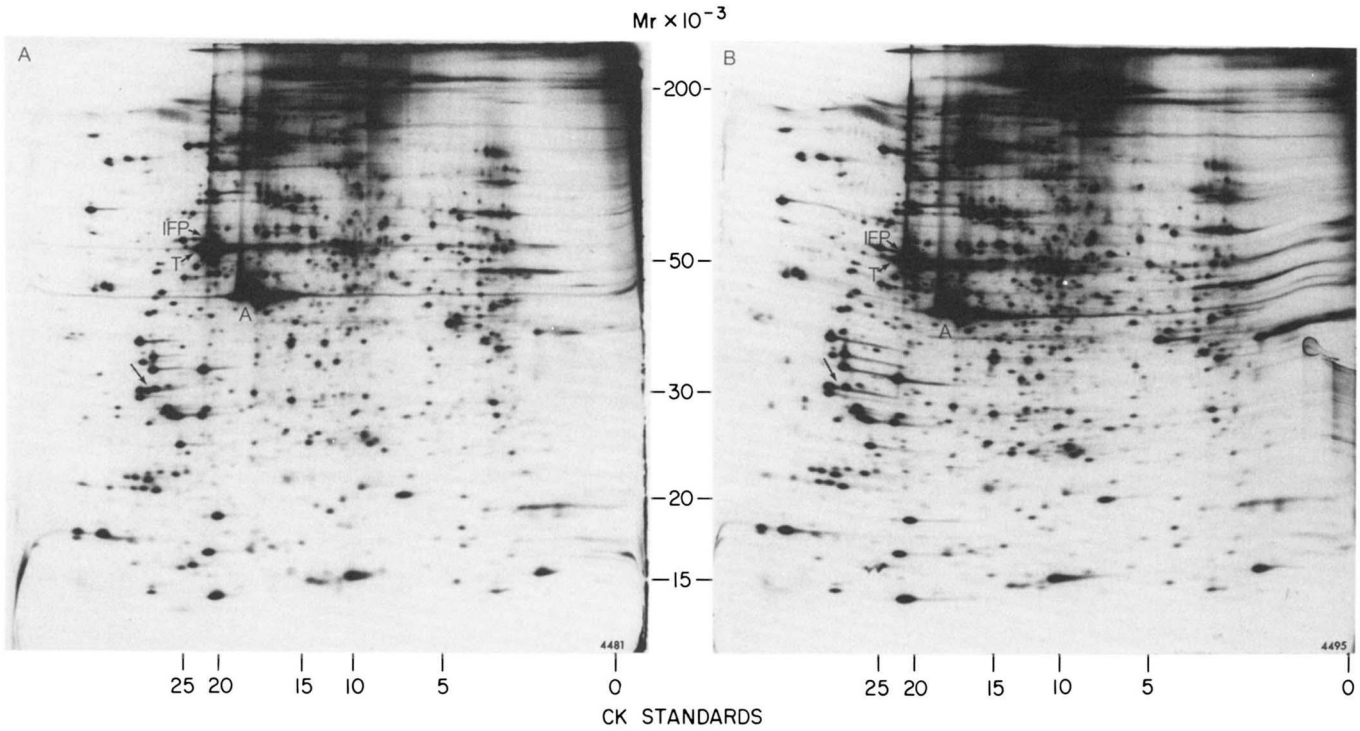

FIG. 1. **Two-dimensional electrophoretic patterns of [^{35}S] methionine-labeled total proteins from cell lines 1493 and 1494.** Samples were prepared, and electrophoresis was performed as described under "Materials and Methods." These patterns are oriented with the acid side to the *left* and the basic side to the *right*. The *horizontal scale* shows the position of carbamylated creatine kinase (*CK*) charge standards used to calibrate the isoelectic focusing dimension (see Ref. 28). The *vertical scale* indicates the molecular weight range of the separated proteins as determined using rat heart standards (see Ref. 29). *A*, actin; *T*, tubulin; *IFP*, intermediate filament protein. The *arrows* indicate the variant protein found in line 1493 (*A*), but missing in line 1494 (*B*) and 10 other fibroblast lines analyzed.

with modifications suggested by Carmon *et al.* (16). The gels were then stained, destained, and fluorographed (14).

Determination of Integrated Spot Densities—Integrated densities of selected spots on autoradiograms or fluorograms were determined using the TYCHO two-dimensional gel analysis system at Argonne National Laboratory (to be described in detail elsewhere). Briefly, this system takes a gel image (scanned on a 100-μm grid using an Optronics P-1000 scanner), smooths it, subtracts background, detects the spots as local maxima after convolution sharpening, and then iteratively optimizes the fit of the spots (represented as two-dimensional Gaussians) to the data (17). The accuracy of the quantitation is in the range of ±10–20%.

RESULTS

When the two-dimensional electrophoretic patterns of the 12 cell lines listed in Table I were compared, approximately 1% (5 spots/500 compared) of the proteins were found to be reproducibly present in some lines but absent in others. This figure is in agreement with other reports (18, 19). A majority of the differences found were among minor spots and were present in more than one cell line. An exception to both of these generalizations, however, was found in cell line 1493. Fig. 1 shows the two-dimensional electrophoretic pattern of [^{35}S]methionine-labeled total proteins from line 1493 (Fig. 1*A*) and line 1494 (Fig. 1*B*), cells from dizygous twins. A new major protein spot with an SDS molecular weight of approximately 30,000 is visible in the 1493 pattern and missing in the 1494 pattern. This protein was consistently present in repeated analyses of line 1493 from the same and subsequent passages, but was absent in repeated analyses of the other 11 cell lines.

The uniqueness of the 1493 variant protein prompted an investigation of its biochemical characteristics. The first step was to determine the subcellular localization of the variant. Nonionic detergent treatment of cell lines 1493 and 1494 showed the 1493 variant protein to be a component of the detergent-resistant cytoskeleton, as are intermediate filament protein, actin, and 17 other major proteins in the same isoelectric focusing region (Fig. 2, *A* and *B*). The protein spots to the left and right of the variant protein in the whole cell pattern (designated Cytosk:11 and Cytosk:13, respectively, according to the Argonne protocol for spot numbering (20)) are also associated with the cytoskeletons of both cell lines 1493 and 1494. When the second dimension of separation was performed using slab gels containing 8 M urea with SDS, both Cytosk:11 and Cytosk:12 (the 1493 variant protein) shifted to a higher molecular weight position relative to that seen when non-urea gels were used (Fig. 2, *C* and *D*). Cytosk:13 was found in the same position with or without urea in both line 1493 and line 1494 patterns.

Further biochemical characterization of Cytosk:12 was obtained by selective amino acid labeling (Fig. 3). In line 1493, Cytosk:11 and :12 labeled moderately well with [^{3}H]phenylalanine and [^{3}H]histidine, although less incorporation relative

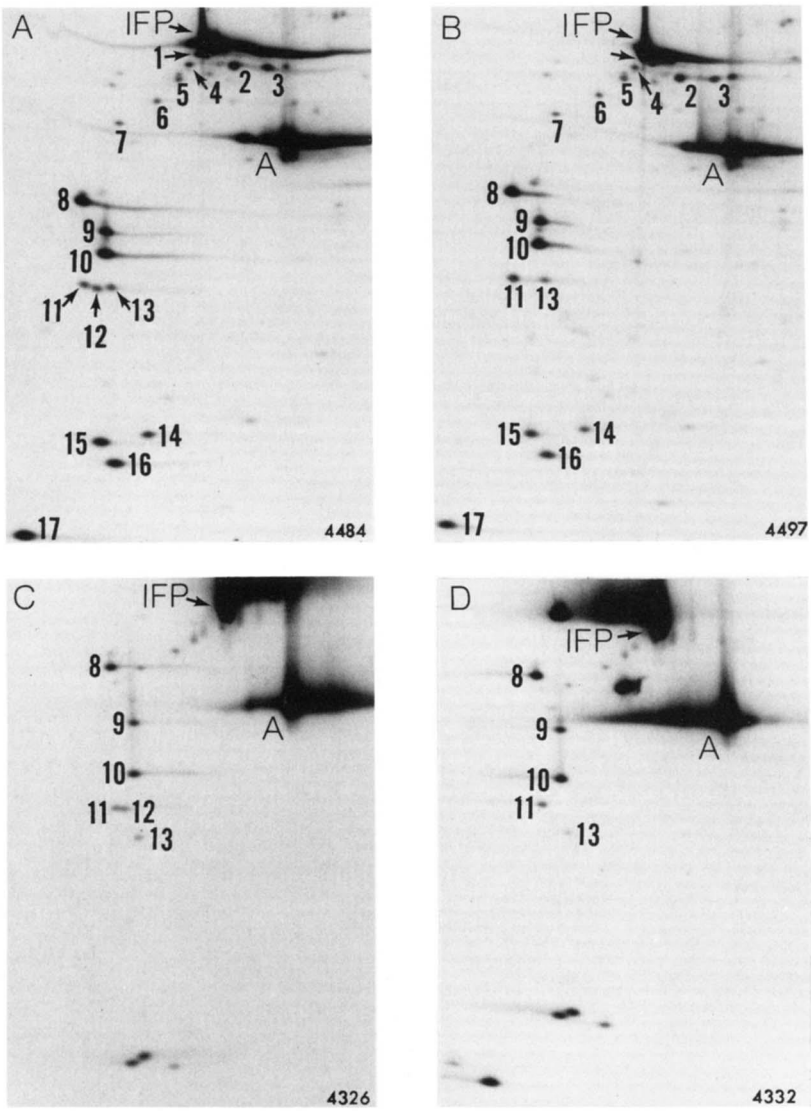

FIG. 2. **Detergent-resistant cytoskeleton proteins from cell lines 1493 and 1494 with the second dimension of separation in the presence or absence of 8 M urea.** Cytoskeletons were prepared as described under "Materials and Methods." The gel pattern orientations and abbreviations are all as described in the legend to Fig. 1. The *numbers* designate major protein spots associated with the cytoskeletons (Cytosk:1–17). After the second dimension of separation in the presence of 0.1% SDS, cytoskeletal proteins from line 1493 include the variant protein, designated Cytosk:12 (*A*). This protein is absent from the 1494 cytoskeleton pattern (*B*). When cytoskeletal proteins were separated in second dimension gels containing 8 M urea in addition to 0.1% SDS, Cytosk:11 and :12 shifted to a higher apparent molecular weight above Cytosk:13 in the 1493 pattern (*C*). Cytosk:11 also shifted to a position above Cytosk:13 in the 1494 pattern (*D*).

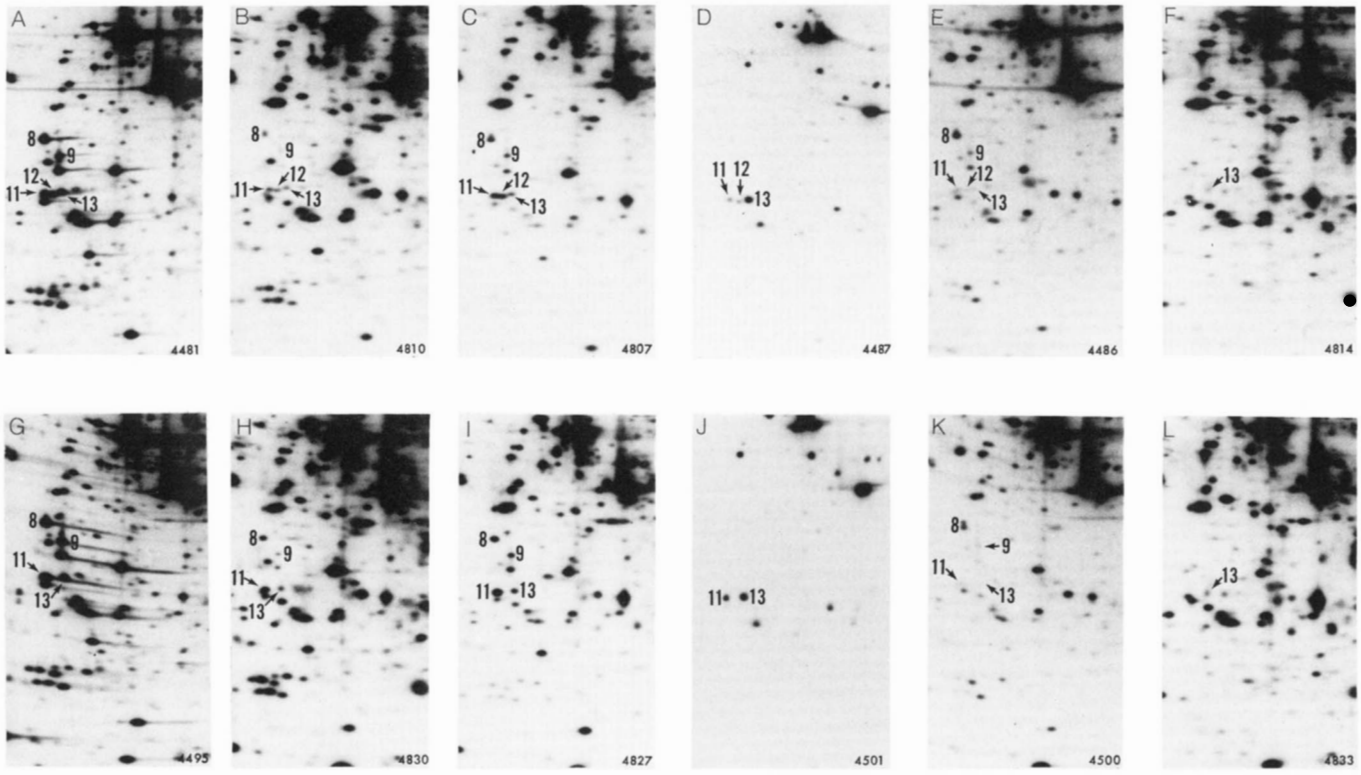

FIG. 3. **Selective amino acid labeling of total cellular proteins from lines 1493 and 1494.** The fibroblasts (*A–F*, 1493; *G–L*, 1494) were labeled as described under "Materials and Methods" using [^{35}S]methionine (*A*, *G*), [^{3}H]phenylalanine (*B*, *H*), [^{3}H]histidine (*C*, *I*), [^{14}C]iodoacetamide (*D*, *J*), [^{3}H]proline (*E*, *K*), or [^{3}H]tryptophan (*F*, *L*). Each pair of autoradiograms or fluorograms was exposed the same length of time (1–2 weeks) for accurate comparison of spot densities. The labeling patterns of Cytosk:11 and :12 in line 1493 were similar with each amino acid used, and the sum of their densities equals that of Cytosk:11 alone in each of the corresponding 1494 patterns (see Table II). Neither Cytosk:11 or :12 was labeled by [^{3}H] tryptophan. Pattern orientations are all as in Fig. 1.

to other spots was observed than with [^{35}S]methionine. More of the labeled phenylalanine and histidine were incorporated into Cytosk:11 in line 1494 than in line 1493, as indicated by the larger spots. Alkylation of proteins from lines 1493 and 1494 using [^{14}C]iodoacetamide showed that Cytosk:11 in both cell lines contains fewer free sulfhydryl groups (cysteine) than does Cytosk:13, but it contains a number comparable to Cytosk:12 in the 1493 pattern. Cytosk:11 in the 1494 line incorporated more of the [^{14}C]iodoacetamide than did the corresponding protein in the 1493 line. [^{3}H]Proline was incorporated to a very small degree into Cytosk:11 and :12 of line 1493 and Cytosk:11 of line 1494. Cytosk:13 of line 1493 is just barely visible after [^{3}H]proline labeling, and Cytosk:13 of line 1494 is not visible at all in the fluorogram shown. No detectable [^{3}H]tryptophan was incorporated into Cytosk:11 of either cell line or into Cytosk:12 of line 1493. Cytosk:13, however, was labeled slightly by [^{3}H]tryptophan.

Quantitative densitometry was done on fluorograms or autoradiograms from samples labeled with histidine, iodoacetamide, and methionine, with the results shown in Table II. The amounts of label incorporated into Cytosk:11 and :12 of the 1493 line are very nearly equal in each case, while the sums of the abundances of Cytosk:11 and :12 in 1493 closely approximate the amounts of Cytosk:11 alone in 1494 (also in each case). These results are consistent with the view that line 1493 represents a heterozygous condition in which one of the two genes normally coding for Cytosk:11 codes instead for the variant Cytosk:12. The quantitative results also clearly show the large differences in amino acid composition between Cytosk:11 and :13, thereby effectively ruling out the possibility that Cytosk:12 is a variant of Cytosk:13.

The similarity in amino acid-labeling patterns of Cytosk:11 and :12 and the observation that both proteins shift to a higher molecular weight position on SDS/urea gels suggest that the two proteins are related. Furthermore, the cytoskeletal localization of both Cytosk:11 and :12, their apparent molecular weight of 30,000, their shift on urea gels, and their amino acid compositions all resemble properties of nonmuscle tropomyosins (3, 21, 22). To determine whether Cytosk:11 and Cytosk:12 are nonmuscle tropomyosins, ethanol extracts of cells from both lines 1493 and 1494 were processed for purification of tropomyosin according to the procedure of Fine *et al.* (2). Mouse brain was used as a protein carrier in order to have visible material for handling through the various purification steps. Fig 4 shows the two-dimensional electrophoresis pattern of the resulting fibroblast protein preparations. The 1493 preparation shows an enrichment of two spots that comigrated with Cytosk:11 and :12, while the 1494 preparation

TABLE II

Integrated densities of the indicated spots on six fluorograms or autoradiograms

Selected autoradiograms or fluorograms from Fig. 3 were analyzed using the TYCHO system (see "Materials and Methods"). Results for each pair of similar gels have been normalized by equating the integrated densities for Cytosk:13. In the line 1493, Cytosk:11 and :12 appear roughly equal for each amino acid, and their sum is very nearly equal to Cytosk:11 alone in line 1494 (the control). Values for Cytosk:8 and 9, present in both cell lines, are included for comparison.

Radiolabel	Cell line	Integrated densities of Cytosk spots:					
		8	9	11	12	13	11 + 12
[^{3}H]Histidine	1493	23	21	37	31	18	68
	1494	29	20	71	0	18	71
[^{14}C]Iodoacetamide	1493	0	0	10	9	43	19
	1494	0	0	22	0	43	22
[^{35}S]Methionine	1493	105	64	73	56	75	129
	1494	107	71	117	0	75	117

contained only one enriched spot that co-migrated with Cytosk:11. In addition to the spots that co-migrate with Cytosk:11 and :12, both the 1493 and 1494 preparations contained several minor protein components. The dark spot directly above Cytosk:11 co-migrated with Cytosk:8. As discussed below, the amino acid labeling profile of this protein suggests that it is not a tropomyosin protein. The other spots do not appear in the two-dimensional patterns from whole cell or cytoskeleton preparations and probably represent protein fragments from high molecular weight proteins sensitive to the organic solvents, heat, or acid precipitation used in the tropomyosin purification procedure. The enrichment of Cytosk:11 by this procedure indicates that this protein has other tropomyosin-like properties in addition to amino acid composition and migration shift in the presence of urea. The co-purification of Cytosk:12 from cell line 1493 at a similar level of enrichment suggests that protein is a nonmuscle tropomyosin variant.

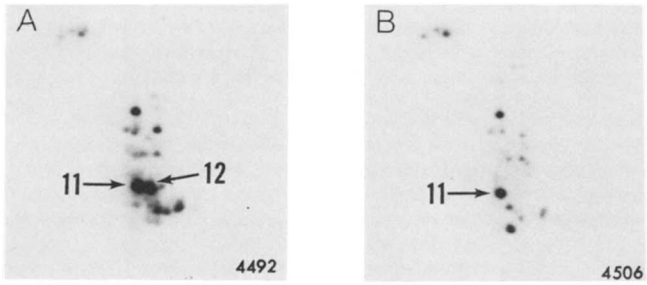

FIG. 4. **Enriched fibroblast tropomyosin from cell lines 1493 and 1494.** Nonmuscle tropomyosin was prepared from ethanol extracts of lines 1493 and 1494 as described by Fine *et al.* (2). The material precipitated with 53% ammonium sulfate was dialyzed, solubilized, and analyzed by two-dimensional electrophoresis as described under "Materials and Methods." The preparation from the cell line 1493 extract (*A*) was enriched in two proteins that co-migrated with Cytosk:11 and :12 in the whole protein pattern of 1493. The 1494 preparation (*B*) yielded one enriched protein that co-migrated with Cytosk:11 in the whole protein pattern of 1494. Pattern orientations are as in Fig. 1.

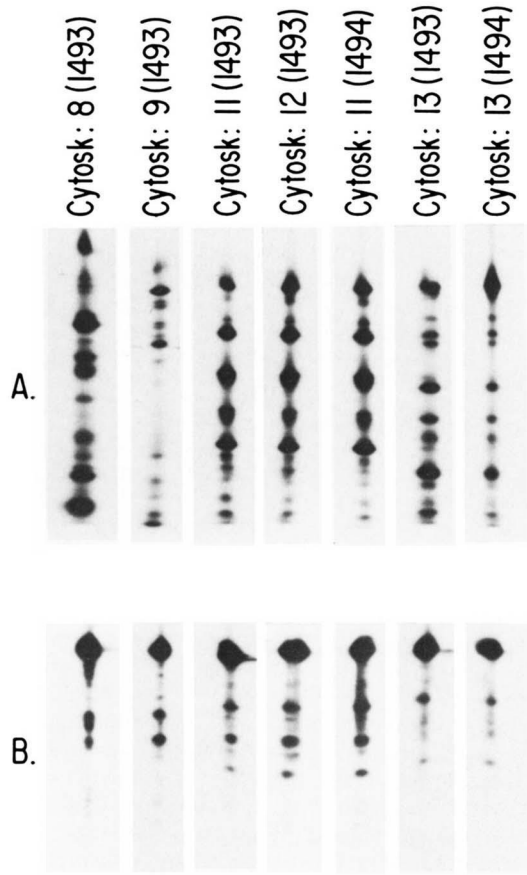

FIG. 5. **Partial proteolysis products of Cytosk:8, :9, :11, :12, and :13 from cell lines 1493 and 1494.** Spots were bored out of dried gels and treated with chymotrypsin (*A*) or trypsin (*B*) as described under "Materials and Methods." The fluorograms, exposed for 7 days, show that partial proteolysis of Cytosk:11 from line 1493 yielded products identical with those of Cytosk:11 from line 1494 and Cytosk:12 from line 1493. The proteolysis products of Cytosk:8 and :9 from line 1493 are different from each other and from the other proteins analyzed. Cytosk:13 from both cell lines yielded patterns identical with each other, but different from Cytosk:11 and :12.

The final verification of the relatedness of Cytosk:12 and Cytosk:11 was obtained using Cleveland gels (15). The spots corresponding to Cytosk:8, :9, :11, :12, and :13 from a gel of proteins from cell line 1493 and the spots corresponding to Cytosk:11 and :13 from a gel of proteins from cell line 1494 were partially digested with chymotrypsin or trypsin. Chymotrypsin cleaves peptide bonds in which the carboxyl group is contributed by an aromatic amino acid, while trypsin cleaves those bonds in which the carboxyl group is contributed by a basic amino acid. The products obtained were separated by SDS electrophoresis and compared. Fig. 5 shows that partial proteolysis of Cytosk:12 from line 1493 with either chymotrypsin (*A*) or trypsin (*B*) produced the same pattern of bands as did Cytosk:11 from both cell lines 1493 and 1494. Cytosk:8, :9, and :13 produced unique band patterns, suggesting that those proteins lack identity with each other and with both Cytosk:11 and :12. The band patterns for Cytosk:13 from both cell lines 1493 and 1494 were identical. Similar results (*i.e.* Cytosk:12 had a band pattern identical with that of Cytosk:11) were obtained using *Staphylococcus aureus* V8 protease (data not shown), an enzyme that cleaves peptide bonds at the COOH-terminal side of aspartic and glutamic acid residues (15).

DISCUSSION

Published work on nonmuscle tropomyosins is very limited, comprising primarily SDS-gel molecular weight determinations, partial amino acid compositions, studies of paracrystal appearance, and assays of actomyosin ATPase activities (1, 2). In addition, there is some evidence that tropomyosin is associated with actin in the microfilaments of nonmuscle cells (1, 22), as might be expected from an analogy with muscle thin filaments. The variant protein Cytosk:12 in line 1493 is associated with the detergent-resistant cytoskeleton of fibroblasts, a structure comprised of both microfilament and microtubule proteins (10, 23), and is therefore expected to contain tropomyosin. The variant protein has a molecular weight of approximately 30,000, similar to reported molecular weights for platelet (1), brain (2), and mouse fibroblast (3) tropomyosins. Two other proteins, Cytosk:11 and Cytosk:13, also have similar molecular weights. However, when the second dimension of separation was done on gels containing urea in addition to SDS, a condition under which tropomyosin is known to have altered migration properties (22), Cytosk:11 and :12 shifted to a higher apparent molecular weight, but Cytosk:13 did not. These results suggested to us that both Cytosk:11 and :12 might be human nonmuscle tropomyosin, with Cytosk:12 being a genetic variant of Cytosk:11.

In addition to Cytosk:11 and :12, Cytosk:8 and :9 showed an obvious shift to a higher apparent molecular weight on the urea/SDS gels. Proteins in similar positions on two-dimensional electrophoresis patterns of L6 myoblasts have also been reported to shift in the presence of urea (24). Garrels (24) suggested that the L6 protein corresponding to Cytosk:8 was

smooth muscle tropomyosin and that the protein corresponding to Cytosk:9 was another form of nonmuscle tropomyosin. Lazarides (21) has reported a fibroblast tropomyosin with a molecular weight of 35,000 that could correspond to Cytosk:8 in our patterns. However, the selective amino acid-labeling data are not consistent with the identification of fibroblast Cytosk:8 and :9 as tropomyosins. Muscle tropomyosin contains no proline (25), and the amino acid compositions for nonmuscle tropomyosins show little or no proline as well (1, 2). Low proline content is essential to the α-helical structure of tropomyosin molecules. Fig. 3, *E* and *K*, shows that both Cytosk:8 and :9 contain appreciable [^{3}H]proline in cell lines 1493 and 1494. Cytosk:9 also labels slightly with [^{3}H]tryptophan, another amino acid not found in rabbit muscle tropomyosin (25). Garrels (24) reported no incorporation of either [^{3}H]proline or [^{3}H]tryptophan into the proteins identified as tropomyosins in cultured myoblasts. Therefore, the amino acid composition data appear to eliminate the possibility that Cytosk:8 and :9 are tropomyosins. The cleavage patterns from Cytosk:8 and :9 obtained using chymotrypsin and trypsin demonstrated that these proteins were totally unrelated to each other or Cytosk:11 and :12. Their shift to a higher molecular weight position on urea/SDS gels probably reflects some chemical commonality with tropomyosin that does not require very low proline and tryptophan contents.

Selective amino acid labeling of cell lines 1493 and 1494 further demonstrates the relatedness of Cytosk:11 and :12. With all six amino acids used, the two proteins incorporate parallel amounts of isotope. As expected for tropomyosin proteins, Cytosk:11 and :12 are both missing in patterns of cells labeled with [^{3}H]tryptophan (23, 24). However, very faint spots are seen in the Cytosk:11 and :12 positions of patterns from cells labeled with [^{3}H]proline. After a 7-day exposure of these fluorograms, no Cytosk:11 or :12 was visible, but after a 14-day exposure, both proteins were just faintly seen. The [^{3}H]proline used was reported by Amersham/Searle to be 95% pure, with the other 5% accounted for as decomposition products, but no other amino acid components. Therefore, the low level of proline incorporation into Cytosk:11 and :12 cannot be explained as contamination of the labeled amino acid. The metabolic pathway of proline in mammals does include conversion to glutamate (25), and both muscle (26) and nonmuscle (1, 2) tropomyosins are reported to contain large amounts of glutamate. Therefore, the small amount of ^{3}H incorporation seen in Fig. 3, *E* and *K*, might be due to a very small percentage of the [^{3}H]proline being converted to glutamate in the cell cultures. However, although the amino acid sequence of rabbit skeletal muscle tropomyosin is known to contain no proline, there are no available sequence data for nonmuscle tropomyosin. The proline content of platelet and brain tropomyosin is reported as 0 and 5 mol/10^5 g, respectively, with the α-helical contents given as 90 and 86% (1, 2). Our data could indicate that the human fibroblast tropomyosin contains a trace amount of proline and therefore differs slightly from muscle tropomyosins. As the interactions between nonmuscle contractile proteins become more clearly understood, the mode of accommodation of this trace proline into the nonmuscle tropomyosin structure should become clear.

The co-purification of Cytosk:11 and :12 using the method outlined for preparation of nonmuscle tropomyosin lends further weight to the identity of these proteins as nonmuscle tropomyosins. Few proteins are stable through ethanol/ether extraction, heating in a boiling water bath, and acid precipitation. Of the proteins seen in Fig. 4, only the two most abundant ones, Cytosk:11 and :12, satisfy all of the tropomyosin-like criteria tested.

The relatedness of Cytosk:11 and :12 demonstrated with Cleveland gels (15) is supported by the quantitation of spots in autoradiograms and fluorograms of cell lines 1493 and 1494. The densitometric scans of these patterns show that Cytosk:11 is present at approximately half of its normal level in the variant 1493 line. However, the sum of the Cytosk:11 and 12 densities from the same 1493 pattern is very nearly equal to the Cytosk:11 density from a 1494 pattern. These quantitative data support the notion that there are normally two genes for Cytosk:11, one of which is altered in 1493 to produce Cytosk:12. Since the individual from whom the original cells for line 1493 were taken has been described as clinically normal, and since 1493 cells grow just as well in culture as 1494, it seems likely that the presence of 50% of the Cytosk:11-type tropomyosin as variant Cytosk:12 molecules has no detrimental effect on normal cellular behavior. The tropomyosin variant was not present in cells from the fraternal twin. Because parental cells were not available for analysis, the possible genetic basis of the tropomyosin variant could not be investigated.

Since tropomyosin is thought to be an evolutionarily stable, conservative protein, the appearance of a tropomyosin variant in normal cells is quite unusual. In addition to 12 human fibroblast lines, we have analyzed 12 fibroblast lines of species ranging from bats to dolphins (data not shown), and in every case, Cytosk:11 is found in the same electrophoretic position as shown for human lines 1493 and 1494. Thus nonmuscle tropomyosin exhibits a strong tendency toward evolutionary charge-conservation. A tropomyosin-like protein occupies a similar position in two-dimensional electrophoretic patterns of human peripheral blood lymphocytes (20). Cytosk:12 has not been found in any of the samples analyzed except for fibroblast line 1493, making this cell line an interesting subject for detailed analysis of cytoskeletal structure and possible alterations due to change in a single protein.

Two-dimensional electrophoresis as described by O'Farrell (7) has a theoretical resolution sufficient to separate over 10,000 protein gene products (9). For this reason, two-dimensional electrophoresis has been suggested as a method for detecting genetic variants within the human population and for assessing human mutation rates (8, 9). When used to analyze the proteins from eukaryotic whole cell homogenates, this technique yields complex patterns of more than 1,000 spots. In such complex patterns, questions arise as to the feasibility of detecting variant proteins and whether or not some identity can be assigned to new protein spots if they are found. Leavitt and Kakunaga (27) recently demonstrated that protein alterations can be detected in two-dimenstional electrophoresis patterns of human cells transformed *in vitro*. In 24 polypeptide differences found, one new spot in the malignant cell patterns was identified as a variant form of actin, another highly conserved contractile protein. In this paper, we have described the detection and characterization of a protein variant found during analysis of normal human fibroblasts by two-dimensional electrophoresis. This observation is important because it is the first report of a variant cytoskeletal protein in normal, untreated human cells. The possibility exists that human nonmuscle tropomyosin is polymorphic, but a large scale screening of many human samples will be required before such a possibility can be confirmed. These reports clearly show, however, that genetic variants can be found and characterized using two-dimensional electrophoresis.

Acknowledgments—We thank J. Taylor, B. Coulter, and A. Scandora, Jr., for their valuable expertise with the TYCHO analysis system. We also wish to thank Dr. Harvey Dosik, who supplied the original biopsies for cell lines 1493 and 1494, and Dr. William Blattner,

who assisted us in obtaining the human fibroblast lines used in this study.

REFERENCES

1. Cohen, I., and Cohen, C. (1972) *J. Mol. Biol.* **68,** 383–387
2. Fine, R. E., Blitz, A. L., Hitchcock, S. E., and Kaminer, B. (1973) *Nat. New Biology* **245,** 182–186
3. Fine, R. E., and Blitz, A. L. (1975) *J. Mol. Biol.* **95,** 447–454
4. Cummins, P., and Perry, S. V. (1973) *Biochem. J.* **133,** 765–777
5. Mak, A. S., Smillie, L. B., and Stewart, G. R. (1980) *J. Biol. Chem.* **255,** 3647–3651
6. Cummins, P., and Perry, S. V. (1974) *Biochem. J.* **141,** 43–49
7. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021
8. Anderson, N. L., and Anderson, N. G. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 5421–5425
9. Anderson, N. G., and Anderson, N. L. (1979) *Behring Inst. Mitt.* **63,** 169–210
10. Osborn, M., and Weber, K. (1977) *Exp. Cell Res.* **106,** 339–349
11. Anderson, N. G., and Anderson, N. L. (1978) *Anal. Biochem.* **85,** 331–340
12. Anderson, N. L., and Anderson, N. G. (1978) *Anal. Biochem.* **85,** 341–354
13. Willard, K. E., and Anderson, N. L. (1980) in *Electrophoresis '79* (Radola, B. J., ed) pp. 415–424, Walter deGruyter, New York
14. Bonner, W. M., and Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 83–88
15. Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) *J. Biol. Chem.* **252,** 1102–1106
16. Carmon, Y., Neuman, S., and Yaffe, D. (1978) *Cell* **14,** 393–401
17. Taylor, J., Anderson, N. L., Coulter, B. P., Scandora A. E., Jr., and Anderson, N. G. (1980) in *Electrophoresis '79* (Radola, B. J., ed) pp. 329–339, Walter deGruyter, New York
18. McConkey, E. H., Taylor, B. J., and Phan, D. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 6500–6504
19. Walton, K. E., Styer, D., and Gruenstein, E. I. (1979) *J. Biol. Chem.* **254,** 7951–7960
20. Anderson, N. L. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 2407–2411
21. Lazarides, E. (1975) *J. Cell Biol.* **65,** 549–561
22. Sender, P. M. (1971) *FEBS Lett.* **17,** 106–109
23. Solomon, F., Magendantz, M., and Salzman, A. (1979) *Cell* **18,** 431–438
24. Garrels, J. I. (1979) *Dev. Biol.* **73,** 134–152
25. Meister, A. (1965) *Biochemistry of the Amino Acid*, Vol. 2, p. 714, Academic Press, New York
26. Dayhoff, M. O. (1978) *Atlas of Protein Sequence and Structure*, Vol. 5, Suppl. 3, p. 278, National Biomedical Research Foundation, Silver Spring, MD
27. Leavitt, J., and Kakunaga, T. (1980) *J. Biol. Chem.* **255,** 1650–1661
28. Anderson, N. L., and Hickman, B. J. (1979) *Anal. Biochem.* **93,** 312–320
29. Giometti, C. S., Anderson, N. G., Tollaksen, S. L., Edwards, J. J., and Anderson, N. L. (1980) *Anal. Biochem.* **102,** 47–58