Proc. Natl. Acad. Sci. USA
Vol. 78, No. 9, pp. 5633–5637, September 1981
Cell Biology

Tropomyosin is decreased in transformed cells

(Rous sarcoma virus/MC29 retrovirus/methylcholanthrene/cytoskeleton/protein blotting)

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Communicated by Edwin G. Krebs, May 15, 1981

ABSTRACT **The steady-state level and synthesis of a pair of polypeptides of M_r 33,000 and 35,000 in chicken embryo fibroblasts (CEF) transformed by Rous sarcoma virus (RSV) are significantly decreased relative to normal CEF; however, the decrease is more pronounced in the case of the M_r 35,000 polypeptide. These polypeptides have been identified as the α and β subunits of CEF tropomyosin by selective staining with tropomyosin antibody, two-dimensional gel electrophoresis, partial peptide analysis, and solubility properties. The decrease in tropomyosin is shown to be a transformation-specific phenomenon in that it does not occur after infection with a virus deleted in *src* sequences. Decreased synthesis of tropomyosin is also observed in quail cells transformed by MC29 (a retrovirus with a different *onc* gene than that in RSV) and also in chemically transformed quail cells. The decrease in tropomyosin is probably not a direct result of the disruption of the microfilament system in transformed cells because disruption of the microfilament system with trypsin or cytochalasin B in normal CEF does not lead to a decrease in tropomyosin synthesis. A decrease in tropomyosin in CEF after transformation may be a result of a pleiotropic effect that results in the transcriptional inactivation not only of the tropomyosin gene but also of the fibronectin and procollagen genes described by others.**

Malignant transformation of cells is characterized by cellular changes in (*i*) growth and hexose transport, (*ii*) adhesiveness and motility, and (*iii*) shape and organization of the cytoskeleton (1–3). Recently, cases have been reported that show changes in genomic transcription after malignant transformation. Transformation of chicken embryo fibroblasts (CEF) by Rous sarcoma virus (RSV) leads to an activation of embryonic globin genes (4) and to an overall increase in genomic transcription corresponding to ≈1000 new transcription units (5). Furthermore, RSV-transformed CEF synthesize less collagen and fibronectin than do normal CEF, and this effect is due to a lower rate of transcription of these genes in transformed CEF (6–10).

There long has been an interest in examining normal and transformed cells for differences in the form or level of cytoskeletal proteins because transformed cells almost always show morphological differences from normal cells, and they are known to have an altered cytoskeleton (3). In this report we show that the steady-state level and the synthesis of tropomyosin, especially the β-subunit, are considerably lower in RSV-transformed CEF than in normal CEF. The decrease in tropomyosin synthesis may play a role in the disruption of the cellular cytoskeleton after transformation.

MATERIALS AND METHODS

Cells and Viruses. CEF were dissected from the region of the developing breast muscle of 11-day-old virus-free chicken embryos (H and N Farms, Redmond, WA) and grown as described (4). In some cases, primary fibroblast cultures were prepared from the whole embryo minus the head and feet. (All fibroblast cultures, regardless of origin, proved to be identical for the purposes of this investigation.) Tertiary cultures were infected with the Prague strain C of RSV and the transformation-defective Prague strain of RSV (td RSV) as described (4). Normal quail cells, quail cells virally transformed by MC29 (Q8), quail cells chemically transformed by methylcholanthrene (QT35) (11), and all viruses were kindly provided by M. Linial. All cells were cultured in plastic tissue culture dishes as described (12) and harvested in logarithmic phase.

Preparation of Cell Extracts. Cells were scraped from tissue culture dishes with a rubber policeman, washed twice with phosphate-buffered saline and washed once with 0.15 M NaCl/0.015 M sodium citrate, pH 7. Whole cell extracts were prepared by lysing cells in either acetic acid/urea gel sample buffer [8 M urea/6% (vol/vol) acetic acid/0.002% 2-mercaptoethanol/2 mg of protamine sulphate per ml (Sigma)] or $NaDodSO_4$ gel sample buffer [0.05 M Tris·HCl, pH 6.8/1% $NaDodSO_4$/1% 2-mercaptoethanol/10% (vol/vol) glycerol] and, for acid/urea extracts, pelleting the insoluble material by centrifugation. For preparation of cytoplasmic extracts, cells were lysed in reticulocyte standard buffer [0.01 M Tris·HCl, pH 7.4/0.01 M NaCl/3 mM $MgCl_2$] containing 0.25% Nonidet P-40 (NP-40) (British Drug House) and 1 mM phenylmethylsulfonyl fluoride. The lysate was centrifuged to pellet nuclei, and the supernatant was lyophilized to dryness and resuspended in gel sample buffer. For preparation of EDTA extracts, the reticulocyte standard buffer/NP-40-insoluble pellet was suspended in 5 mM EDTA, pH 7/1 mM phenylmethylsulfonyl fluoride, mixed, and recentrifuged to a pellet. The supernatant was then lyophilized to dryness and resuspended in gel sample buffer. Contents of the EDTA-insoluble pellet were analyzed by suspending this pellet directly in gel sample buffer followed by electrophoresis.

Labeling of Cells. Cells were pulse-labeled for 3 or 15 min at 37°C with [^{35}S]methionine (a gift from R. Eisenman) at 30 μCi/ml (1 Ci = 3.7×10^{10} becquerels) in methionine-free minimal essential medium containing 2.5% (vol/vol) dialyzed calf serum. Then the cells were extracted. For experiments involving trypsin treatment prior to labeling, cells were treated with 0.05% trypsin for 5 min as described (12) and maintained as round cells by gentle gyration at 37°C in a sterile plastic tube for 7 hr prior to the addition of [^{35}S]methionine. In experiments involving cytochalasin B, cells were incubated with cytochalasin B at a concentration of 2 μM as described (13) for 2 hr prior to the addition of [^{35}S]methionine. The same concentration of cytochalasin B was maintained during the labeling period.

Gel Electrophoresis. Slab gel electrophoresis was conducted with a minigel apparatus (8 × 10 cm plates; Idea Scientific, (Corvallis, OR) as described (14). $NaDodSO_4$ gel electropho-

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Abbreviations: CEF, chicken embryo fibroblasts; RSV, Rous sarcoma virus; td RSV, transformation-defective Prague strain of RSV; rTM, rabbit skeletal muscle tropomyosin; NP-40, Nonidet P-40; P_i/NaCl, phosphate-buffered saline.

resis was performed with the stacking gel and discontinuous buffer system described by Laemmli (15); discontinuous acetic acid/urea gel electrophoresis (16) and two-dimensional gel electrophoresis (17) were as described.

Samples for $NaDodSO_4$ gels were suspended in 0.05 M Tris·HCl, pH 6.8/1% $NaDodSO_4$/1% 2-mercaptoethanol/10% (vol/vol) glycerol and were briefly heated to 100°C before applying to gels. Samples for acid/urea gels were suspended in 8 M urea/6% (vol/vol) glacial acetic acid/0.002% 2-mercaptoethanol/2 mg of protamine sulfate per ml and were briefly heated to 37°C before applying to gels. Minigels were run at 130 V for 2–3 hr. After electrophoresis, the gels were fixed and stained for protein for 20 min in 50% (vol/vol) methanol/10% glacial acetic acid/0.25% Coomassie brilliant blue R, destained in 10% methanol/10% acetic acid, and then, in the case of labeled samples, treated for fluorography with EN^3HANCE (New England Nuclear). After being dried, the gels were exposed to Kodak XR-5 x-ray film at −70°C. Gels and x-ray films were scanned with a Zeineh soft laser scanning densitometer.

Gel Blotting and Antibody Labeling of Proteins. Proteins were transferred from polyacrylamide gels to nitrocellulose by diffusion as described (18). Two nitrocellulose replicas of each gel were made, one of which was stained with 0.1% aniline blue black/10% acetic acid/43% methanol, and the other was used for antibody labeling. Antibody prepared to tropomyosin from the smooth muscle of chicken gizzard was a gift from J. Croop and H. Holtzer. Authentic rabbit skeletal muscle tropomyosin (rTM) was a gift from E. Fischer. The procedure used for antibody labeling of the filter was a modification of that described by others (19, 20). The nitrocellulose replicas were placed into phosphate-buffered saline (P_i/NaCl; 10 mM Na phosphate, pH 7.4/0.1 M NaCl) containing 5% (vol/vol) bovine serum albumin for 2 hr at 37°C. Filters were then rinsed in P_i/NaCl and incubated overnight at 4°C in P_i/NaCl/5% albumin containing antiserum diluted 1:100. To remove unreacted antibody, filters were washed once at room temperature in P_i/NaCl for 10 min and twice in P_i/NaCl/0.05% NP-40, followed by a brief rinse with P_i/NaCl again. Filters were then placed into P_i/NaCl/5% albumin containing ^{125}I-labeled *Staphylococcus aureus* protein A (10^5 cpm/ml; provided by C. Kane) for 4 hr at 4°C. Unbound ^{125}I-labeled protein A was washed free of the filters by washing first in P_i/NaCl, then in P_i/NaCl/0.05% NP-40, and finally in P_i/NaCl again. Filters were blotted dry on Whatman 3MM paper, covered with Saran Wrap, and autoradiographed at −70°C using Kodak XR-5 x-ray film with an intensifying screen.

RESULTS AND DISCUSSION

Decrease in Tropomyosin in RSV-Transformed CEF. When CEF grown in culture are extracted with nonionic detergents such as NP-40 or Triton X-100, the insoluble material that remains include nuclei and the cellular cytoskeleton (21–23). When this insoluble residue was extracted with 5 mM EDTA (pH 7), the major proteins that were solubilized (Fig. 1*C*) included a protein of M_r 45,000, identified as actin by comigration with authentic actin in $NaDodSO_4$ gels (data not shown), and two polypeptides of M_rs 33,000 and 35,000, putatively identified as CEF tropomyosin.

The M_r 33,000 and 35,000 putative tropomyosin subunits were more readily visualized if the 5 mM EDTA cytoskeletal extracts of normal CEF and CEF transformed by RSV were analyzed on a discontinuous acetic acid/urea gel system (16), which gave excellent resolution. In addition, because most of the extracted proteins migrated toward the anode under these conditions, the gels also gave a striking enrichment for tropomyosin, which migrated toward the cathode as demonstrated with rTM (not shown). Fig. 1*A* shows that there is a dramatic difference between the relative amounts of tropomyosin (indicated by arrows) in normal cells and in RSV-transformed cells. No reproducible differences were observed in the amount of actin extracted by this procedure or in the amount of actin in whole cell extracts.

Relative to overall protein content, the amount of the faster migrating tropomyosin subunit (α) from the transformed cells was reproducibly less than half the amount of the α subunit from the normal cells as determined by densitometry. More striking, however, is the observation that the slower migrating subunit (β) was virtually absent in the transformed cells—only trace amounts being detectable by staining. The relative difference in the amount of tropomyosin subunits between normal and RSV-transformed cells was reflected also in the protein pattern on $NaDodSO_4$ gels (Fig. 1*C*). Two-dimensional gels (acid/urea in the first dimension and $NaDodSO_4$ in the second) indicated that the α and β bands of the acid/urea gels correspond to the α and β tropomyosin bands as identified on the $NaDodSO_4$ gels (not shown). In Fig. 1*A*, the overall protein patterns, excluding the two tropomyosin bands, of the normal CEF and RSV-transformed CEF extracts appear very similar. This tends to argue against cell-type specific proteolysis contributing to the loss of tropomyosin in the RSV-transformed cells. Also in support of this argument is the finding that when RSV-transformed CEF and normal CEF were mixed prior to lysis and extraction, the ratio of the intensities of the β and α subunits in the mixture was exactly what was expected by the simple addition of the band intensities in the separate extracts (not shown).

It is possible that the amount of tropomyosin in the RSV-transformed cells is equal to that in normal cells, but tropomyosin is not quantitatively extracted with 5 mM EDTA. However, analysis of *all* cellular fractions, generated during the preparation of the EDTA extract, showed this not to be the case (not shown). In support of this conclusion, when whole cells (normal and RSV-transformed CEF) were dispersed in acetic acid/urea sample buffer and equal amounts of material were analyzed on an acetic acid/urea gel (Fig. 1*B*), again it was clear—at least in the case of the more easily resolved β subunit—that tropomyosin was considerably reduced in the transformed cells. A similar result was also obtained when whole cell extracts were resolved on $NaDodSO_4$ gels and tropomyosin was stained with tropomyosin antiserum.

Synthesis of the α and β Polypeptides. The difference in the steady-state amount of tropomyosin (as revealed by Coomassie

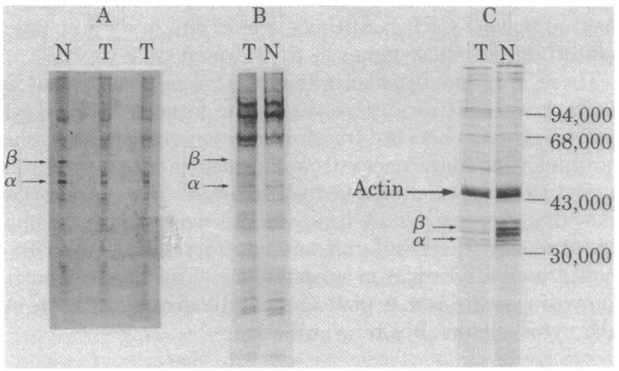

FIG. 1. Gel electrophoresis of EDTA-soluble proteins and whole cell extracts. All gels are stained with Coomassie blue. (*A*) Gel (15% acetic acid/urea) containing EDTA-soluble proteins from the NP-40-insoluble pellet of cells. The T sample was run in duplicate. (*B*) Gel (15% acid/urea) containing proteins solubilized from whole cells by gel sample buffer. (*C*) Gel (15% $NaDodSO_4$) containing the same protein samples as applied to gel *A*. N, normal cells; T, transformed cells; α and β, two subunits of tropomyosin (24, 25). α-Tropomyosin is not easily resolved from contaminating proteins by Coomasie blue staining of the $NaDodSO_4$ gel shown in *C* (see Fig. 4).

blue staining) between normal CEF and RSV-transformed CEF may be a result of different levels of synthesis or, alternatively, different turnover rates. To examine these alternatives, cells were pulse-labeled with [^{35}S]methionine and whole cell extracts and EDTA extracts of the NP-40-insoluble preparations were analyzed on acetic acid/urea gels.

Fig. 2 shows autoradiograms of acid/urea gels containing labeled EDTA extracts and labeled whole cell extracts of normal and RSV-transformed CEF. Fig. 2*B* is the autoradiogram of the Fig. 1*A* stained gel, and Fig. 2*D* is the autoradiogram of the Fig. 1*B* stained gel. The stained gels of Fig. 1 *A* and *B* show that the overall amount of protein in both the normal and RSV-transformed CEF samples was about equal; therefore, the differences in band intensities between the normal and RSV-transformed CEF samples in the autoradiograms of Fig. 2 cannot be accounted for by differences in loading. The autoradiogram of the gel shown in Fig. 1*A* (i.e., Fig. 2*B*) shows that during a 15-min pulse-labeling, the overall synthesis of the α and β subunits of tropomyosin was much lower in transformed cells than in normal cells. Also, β synthesis was considerably less than α synthesis in transformed cells—in agreement with the stained pattern. When cells were labeled for only 3 min (Fig. 2*A*), again it appeared that the level of synthesis of tropomyosin was considerably less in the transformed cells. The decrease in β-tropomyosin synthesis also could be seen in whole cell extracts after a 3-min pulse (Fig. 2*D*). (Because of comigrating proteins, it was difficult to resolve the α-subunit in whole cell extracts.) Therefore, it is unlikely that degradation of tropomyosin after synthesis in transformed cells can account for the reduced levels of the protein present in these cells, but rather most of the decrease in levels of tropomyosin in RSV-transformed CEF probably results from a decrease in the rate of tropomyosin synthesis.

The tropomyosin pattern of CEF infected with td RSV was also examined. This virus replicates normally in CEF, but because of a deletion in the *src* gene, transformation does not occur. The synthesis pattern in Fig. 2*C* shows that the relative amount of each tropomyosin subunit synthesized in td RSV cells was virtually identical to that in the normal cells. This suggests that the decrease in the relative amount of tropomyosin in RSV-transformed CEF is a phenomenon resulting from the transformation of the cell and not from viral infection *per se*. The tropomyosin content of chemically transformed quail cells (QT35) was also considerably lower than that of normal quail cells, lending further support to the belief that this phenomenon is due to a transformation-specific effect.

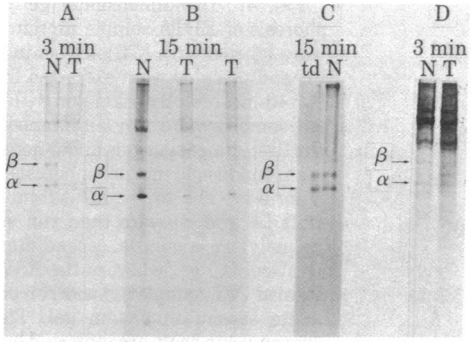

FIG. 2. Synthesis of α and β polypeptides. Shown are autoradiograms of 15% acetic acid/urea gels. Cells were labeled with [^{35}S]methionine for the times indicated, and proteins of the cellular fractions were analyzed. (*A–C*) EDTA-soluble proteins from the NP-40-insoluble pellet of cells. (*B*) Autoradiogram of the stained gel shown in Fig. 1*A*. (*D*) Proteins solubilized from whole cells by gel sample buffer; autoradiogram of the stained gel shown in Fig. 1*B*. N, normal cells; T, transformed cells; td, cells infected with td RSV. Tropomyosin α and β subunits are indicated by arrows.

Identification of the α and β Polypeptides as Tropomyosin. The α and β polypeptides were present along with a considerable amount of actin in 5mM EDTA extracts of pellets remaining after lysing cells with reticulocyte standard buffer/NP-40 (Fig. 1*C*). Because the NP-40-insoluble material is known to be enriched in cytoskeletal components (21, 22), this observation together with their sizes, quantity, and solubility properties suggested that these two polypeptides (α and β) could be the α and β subunits of tropomyosin. This hypothesis was tested directly by using antiserum prepared against chicken gizzard tropomyosin (provided by J. Croop) to label protein transferred to nitrocellulose from acrylamide gels (18). Fig. 3 shows a $NaDodSO_4$ gel of whole cells and an acid/urea gel of EDTA-extracted NP-40 lysates stained with Coomassie blue. The figure also shows the corresponding autoradiograms after staining with the tropomyosin antiserum and labeling with ^{125}I-labeled *S. aureus* protein A. The specificity of the rabbit antiserum obtained against chicken gizzard tropomyosin has been tested by double immunodiffusion and was found to crossreact with preparations of chicken skeletal muscle tropomyosin, chicken fibroblast tropomyosin, and rTM (J. Croop, personal communication). The autoradiogram of the $NaDodSO_4$ (Fig. 3*A*) verifies that the tropomyosin antiserum crossreacted quite well with rTM, which migrate somewhat more slowly than did chicken tropomyosin (27). The reaction was more intense with the β-subunit of rTM as seen by comparison with the stained gel. A similar preference for the β-subunit was also seen with

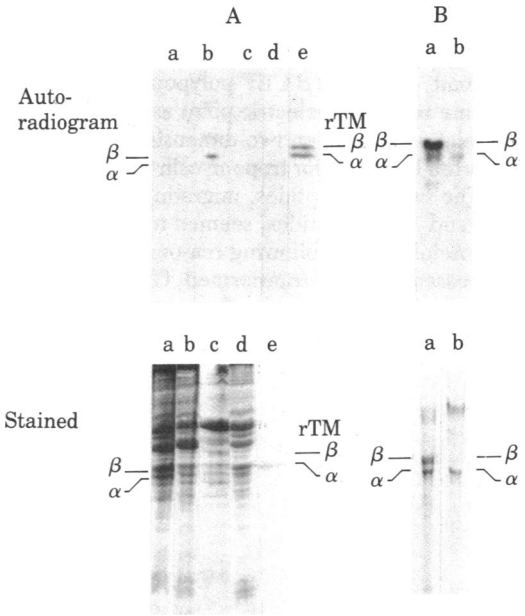

FIG. 3. Labeling of α and β tropomyosin by selective reaction with tropomyosin antibody. Protein samples were electrophoresed on $NaDodSO_4$ or acetic acid/urea gels, transferred to nitrocellulose, and labeled with antibody and ^{125}I-labeled *S. aureus* protein A. Duplicate gels were run so that one was stained with Coomassie blue and the other used for protein blotting onto nitrocellulose and labeling with antibody. (*A*) $NaDodSO_4$ gel containing proteins solubilized by gel sample buffer from whole cells of RSV-transformed CEF (lane a), td-RSV infected CEF (lane b), MC29 transformed quail cells (Q8) (lane c), and chemically transformed quail cells (QT35) (lane d). Lane e shows rabbit skeletal muscle tropomyosin (rTM), which migrates slightly slower than CEF tropomyosin. The same overall staining pattern is obtained whether whole antiserum (lanes a–e) or total IgG, purified by affinity chromatography (26), is used (not shown). The crossreacting proteins migrating slower than tropomyosin also appear to be decreased in RSV-transformed cells. (*B*) Acid/urea gel containing proteins solubilized by 5 mM EDTA from the NP-40-insoluble pellet of normal CEF (lane a) and RSV-transformed CEF (lane b). The positions of the α and β CEF tropomyosin subunits are indicated by arrows.

chicken tropomyosin. In the $NaDodSO_4$ gel (Fig. 3A) many proteins were apparent after Coomassie blue staining; however, the antiserum to tropomyosin selectively reacted with the two polypeptides (indicated by arrows) that migrated as did the α and β proteins previously shown to be decreased in RSV-transformed cells. The reaction of the antibody with these proteins from RSV-transformed cells was greatly decreased. Several crossreacting proteins, migrating slower than tropomyosin, were also stained by the antibody under these denaturing conditions. The amount of these proteins also appeared to be decreased in RSV-transformed cells. A comparison of the Coomassie blue-stained acid/urea gel with its corresponding autoradiogram (Fig. 3B) shows that the tropomyosin antiserum reacted quite strongly with the two polypeptides (indicated by arrows) described in the preceding sections. Here, again, the reaction was much stronger with the larger (β) subunit. Duplicate experiments with nonimmune serum (not shown) showed no reaction with these proteins.

The α and β polypeptides described here were characterized further by two-dimensional gel electrophoresis (fig. 4). Normal CEF and RSV-transformed CEF were labeled with [^{35}S]methionine for 14 hr and extracted with EDTA. To half of the sample from normal CEF, 10 μg of unlabeled rTM was added as an internal marker (Fig. 4A). Among the major proteins in the EDTA extract are actin and the putative CEF tropomyosin. The α-subunit of rTM comigrated with the β-subunit of the putative CEF tropomyosin (Fig. 4A). This was also seen in the blot of the one-dimensional $NaDodSO_4$ gel (Fig. 3). The putative CEF tropomyosin (especially the β-subunit) was considerably decreased in the RSV-transformed CEF (Fig. 4C). Most important, the α and β CEF polypeptides showed essentially the same relative isoelectric point as did rTM, and their migration relative to actin on two-dimensional gels was similar to that reported by others for tropomyosin from chicken muscle cells (28). The two polypeptides, migrating slightly faster than the CEF α and β polypeptides, seemed to be unrelated to the α and β proteins for the following reasons: they appeared not to be decreased in RSV-transformed CEF (Fig. 4C); they seemed to be methionine-poor after steady-state labeling as compared to the α and β polypeptides (see autoradiograms); and they were not labeled with tropomyosin antibody (Fig. 3).

Thus, by their solubility properties; mobilities on acid/urea gels, $NaDodSO_4$ gels, and two-dimensional gels; and by their reaction with tropomyosin antibody, we conclude that the two proteins depleted in transformed cells are α and β tropomyosin. Additional data from partial peptide analysis (29) (not shown) support this conclusion.

The $NaDodSO_4$ gel blot (Fig. 3A) shows that in a whole cell extract solubilized by $NaDodSO_4$, antibody-stained tropomyosin is significantly reduced in RSV-transformed CEF relative to td-RSV cells. The antibody reactions were performed in antibody excess (i.e., doubling the antibody concentration in the presence of excess ^{125}I-labeled *S. aureus* protein A resulted in no increase in signal), and under protein concentrations such that doubling the amount of protein loaded onto the gels resulted in a doubling of the antibody signal. Therefore, the intensities of the bands on the blot provided a direct and roughly quantitative measurement of the amount of antigen present, given that each sample contained the same amount of protein.

The two tropomyosin subunits are also greatly reduced in chemically transformed quail cells (QT35) (Fig. 3A). Normal quail cells (not shown) showed essentially the same relative amounts of tropomyosin as normal or td RSV chicken embryo fibroblasts. As seen here, the tropomyosin decrease observed in chemically transformed cells supports the previous results suggesting that the decrease in tropomyosin in CEF is a transformation-specific phenomenon. A similar decrease in tropomyosin was also observed in fibroblasts transformed by MC29 (Fig. 3A, lane c), a virus that contains an *onc* gene that is different from the *onc* gene (i.e., *src*) of RSV (30).

Mechanism of Tropomyosin Decrease. To gain some insight into the mechanism by which tropomyosin synthesis is decreased in RSV-transformed CEF, we asked if this decrease could simply be a result of or correlated with the rounded appearance characteristic of transformed cells. (This is somewhat unlikely because although RSV-transformed CEF are quite round and detached, chemically transformed quail cells are elongated and firmly attached and MC29-transformed quail cells are "fusiform".) To address this question, we treated normal CEF with trypsin, and the resulting "rounded" cells were

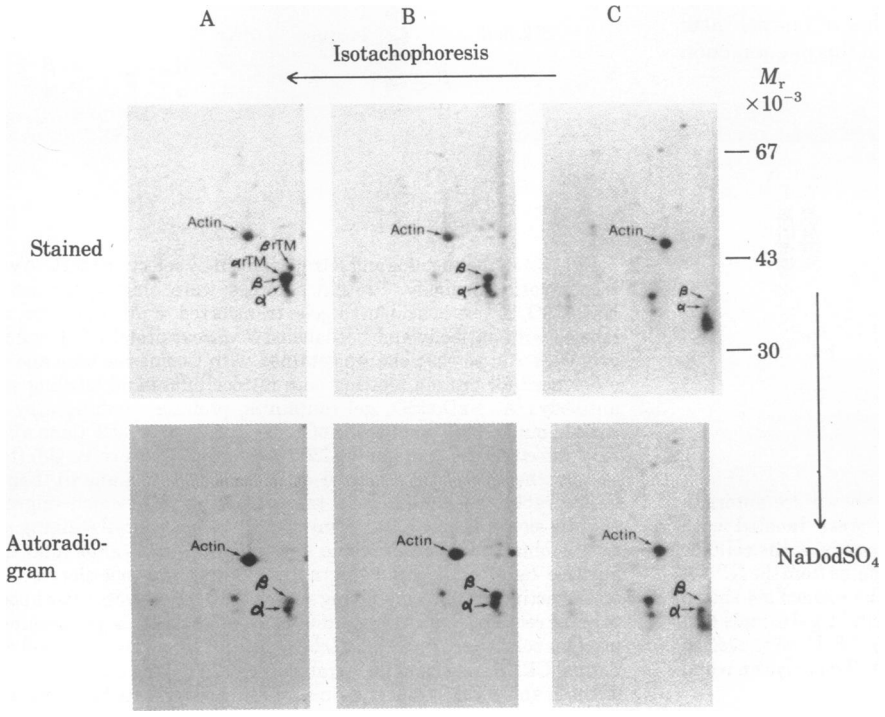

FIG. 4. Two-dimensional gel electrophoresis of EDTA-soluble proteins. Cells were labeled with [^{35}S]methionine for 14 hr, and proteins were extracted from the NP-40-insoluble cytoskeleton with EDTA. All samples were run simultaneously on the first-dimension gels under nonequilibrium conditions (isotachophoresis) at 500 V for 5 hr. The two samples from normal cells (*A* and *B*) were then run simultaneously on a single second-dimension ($NaDodSO_4$) gel whereas the RSV-transformed CEF sample (*C*) was run on a separate second-dimension gel. The CEF tropomyosin spots are indicated by arrows labeled α and β. (*A*) Normal CEF with 10 μg of unlabeled rTM. (*B*) Normal CEF. (*C*) RSV-transformed CEF. Rabbit skeletal muscle tropomyosin alone migrates in these two-dimensional gels in the same position as shown in *A*. The faintness of β in *A* is probably due to quenching by the stained α component of rTM. Molecular weight standards are indicated only for the gel in *C*.

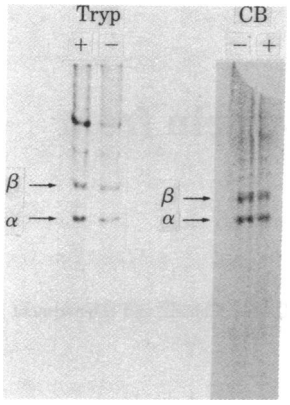

FIG. 5. Synthesis of α and β tropomyosin in "rounded-up" CEF. Normal CEF were labeled with [^{35}S]methionine for 15 min after cells were treated with either trypsin (Tryp; 4 hr) or cytochalasin B (CB; 2 hr). Shown are autoradiograms of 15% acid/urea gels containing proteins solubilized by 5 mM EDTA from the NP-40-insoluble pellet of the above cells. +, Trypsin or cytochalasin B added; −, no addition.

kept in suspension by slow gyration in a sterile plastic tube for 7 hr prior to labeling with [^{35}S]methionine to measure the rate of synthesis of tropomyosin. In other experiments, we treated normal CEF with cytochalasin B for 2 hr prior to the addition of label. Both trypsin and cytochalasin B caused normal cells to "round up" in much the same way as RSV-transformed cells do. In both cases (as in RSV-transformed CEF), the microfilament bundles of the cytoskeleton are also disrupted (13, 31). Equivalent samples from equal numbers of cells were used for comparing the effects of trypsin and cytochalasin B. The level of tropomyosin synthesis in the cells rounded up by treatment with trypsin or cytochalasin B was not significantly different from that in the untreated CEF (Fig. 5). Also, the relative ratio between the β and α subunits did not differ significantly in the rounded-up cells from that in the untreated CEF. This result suggests that when CEF are transformed by RSV, there is not necessarily a causal relationship between the change in cellular shape and the observed decrease in the synthesis of tropomyosin.

The previous experiments suggest that the decrease in tropomyosin synthesis observed in RSV-transformed CEF is not a result of a "feedback" mechanism secondary to the disruption of the cytoskeleton after trypsin or cytochalasin B treatment. Such a mechanism has been demonstrated for tubulin synthesis after disruption of microtubules by colchicine (32). On the other hand, because it is not presently clear that either trypsin treatment or cytochalasin B lead to an increase in free tropomyosin subunits (nor is it clear that RSV transformation leads to an increase in free tropomyosin subunits), the analogy with the tubulin control circuit may not be strictly correct. Nevertheless, at present we think it more likely that the decrease in tropomyosin synthesis may result from a direct inhibition of tropomyosin gene transcription as a consequence of transformation. This inhibition may be analogous to the decrease in procollagen gene transcription and fibronectin gene transcription in RSV-transformed CEF (9, 10), possibly as part of a pleiotropic transcriptional program effected by transformation. This speculation can be tested directly once recombinant DNA clones containing the tropomyosin gene are isolated.

Paulin *et al.* (33) have shown that tropomyosin is undetectable in mouse embryonal carcinoma cells; however, after treatment with hexamethylenebisacetamide, the cells lose their transformed phenotype, differentiate, form actin microfilament bundles, and turn on the synthesis of tropomyosin. The authors interpreted their results to indicate that tropomyosin synthesis was activated by differentiation. In the light of the results presented here, it seems equally likely that tropomyosin synthesis was activated by the loss of the transformed phenotype. We found a similar effect when CEF infected with a temperature-sensitive strain of RSV are shifted from the permissive to the restrictive temperature (results not shown). At the restrictive temperature, these cells lose their transformed phenotype and significantly increase the synthesis of tropomyosin.

Finally, there is the question of the relationship, if any, between a decrease in tropomyosin and the disruption of the actin cables in transformed cells. It is possible that the elongated tropomyosin molecules (34) may be required for the stable assembly of actin filaments and that in transformed cells the absence of actin filaments may result, primarily, from the inactivation of synthesis of tropomyosin, particularly the β subunit.

We thank Drs. J. Croop and H. Holtzer for tropomyosin antiserum and Dr. E. Fischer for rabbit skeletal muscle tropomyosin. We also thank Dr. M. Groudine, Dr. R. Margolis, and Dr. L. Rohrschneider for their helpful comments. This work was supported by a grant from the National Institutes of Health to H.W. and a postdoctoral fellowship award from the National Institutes of Health to M.H.

1. Hanafusa, H. (1977) in *Comprehensive Virology*, eds. Fraenkel-Conrat, H. & Wagner, R. P. (Plenum, New York), Vol. 10, pp. 401–483.
2. Erikson, R. L., Purchio, A. F., Erikson, E., Collett, M. S. & Brugge, J. S. (1980) *J. Cell Biol.* **87**, 319–325.
3. Pollack, R., Osborn, M. & Weber, K. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 994–998.
4. Groudine, M. & Weintraub, H. (1978) *Proc. Natl. Acad. Sci. USA* **72**, 4464–4468.
5. Groudine, M. & Weintraub, H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5351–5354.
6. Bornstein, P. & Palmiter, R. D. (1978) *Biochemistry* **17**, 1581–1590.
7. Howard, H. B., Adams, S. L., Sobel, M. E., Pastan, I. & deCrombrugghe, B. (1978) *J. Biol. Chem.* **253**, 5869–5874.
8. Sandmeyer, S. & Bornstein, P. (1979) *J. Biol. Chem.* **254**, 4950–4953.
9. Sandmeyer, S., Gallis, B. & Bornstein, P. (1981) *J. Biol. Chem.* in press.
10. Fagan, J. B., Sobel, M. E., Yamada, K. M., deCrombrugghe, B. & Pastan, I. (1981) *J. Biol. Chem.* **256**, 520–521.
11. Moscovici, G., Giovannella, M., Jimenez, H., Lai, M. M. C., Hayman, M. J. & Vogt, P. K. (1977) *Cell* **11**, 95–103.
12. Groudine, M., Das, S., Neiman, P. & Weintraub, H. (1978) *Cell* **14**, 865–878.
13. Weber, K., Rathke, P., Osborn, M. & Franke, W. (1976) *Exp. Cell Res.* **102**, 285–297.
14. Matsudaira, P. T. & Burgess, D. R. (1978) *Anal. Biochem.* **87**, 386–396.
15. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
16. Bonner, W. M., West, M. H. P. & Stedman, J. D. (1980) *Eur. J. Biochem.* **109**, 17–23.
17. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P H. (1977) *Cell* **12**, 1133–1142.
18. Bowen, B., Steinberg, J., Laemmli, U. K. & Weintraub, H. (1980) *Nucleic Acids Res.* **8**, 1–20.
19. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
20. Renart, J., Reiser, J. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3116–3120.
21. Brown, S. Levinson, W. & Spudich, J. A. (1976) *J. Supramol. Struct.* **5**, 119–130.
22. Osborn, M. & Weber, K. (1977) *Exp. Cell Res.* **106**, 339–349.
23. Webster, R. S., Henderson, D., Osborn, M. & Weber, K. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5511–5515.
24. Cummins, P. & Perry, S. V. (1973) *Biochem. J.* **133**, 765–777.
25. Cummins, P. & Perry, S. V. (1974) *Biochem. J.* **141**, 43–49.
26. Goudswaard, J., Van der Donk, J. A., Noordzij, A., Van Dam, R. H. & Vaerman, J. P. (1978) *Scand. J. Immunol.* **8**, 21–28.
27. Lazarides, E. (1975) *J. Cell Biol.* **65**, 549–561.
28. Izant, J. G. & Lazarides, E. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1450–1454.
29. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106.
30. Stahelin, D. & Graf, T. (1978) *Cell* **13**, 745–750.
31. Heuser, J. E. & Kirschner, M . W. (1980) *J. Cell Biol.* **86**, 212–234.
32. Ben-Ze'ev, A., Farmer, S. R. & Penman, S. (1979) *Cell* **17**, 319–325.
33. Paulin, D., Perreau, J., Jakob, H., Jacob, F. & Yaniv, M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1891–1895.
34. Casper, D. L. D., Cohen, C. & Longley, W. (1969) *J. Mol. Biol.* **41**, 87–107.