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Suppression of Tropomyosin Synthesis, a Common Biochemical Feature of Oncogenesis by Structurally Diverse Retroviral Oncogenes

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To identify proteins whose production may be altered as a common event in the expression of structurally diverse oncogenes, we compared two-dimensional electropherograms of newly synthesized proteins from NIH/3T3 cell lines transformed by a variety of retroviral oncogenes, from cellular revertant lines, and from a line (433.3) which expresses the v-*ras* oncogene in response to corticosteroids. Most alterations in the synthesis of specific proteins detected by this approach appeared to be the result of selection during prolonged cultivation and were probably unrelated to the transformation process. However, we detected seven proteins whose synthesis was strongly suppressed in cell lines transformed by each of the six retroviral oncogenes we studied and whose production was fully or partially restored in two cellular revertant lines. Suppression of two of these proteins was also correlated with the initial appearance of morphological alteration during corticosteroid-induced oncogene expression in 433.3 cells. These proteins (p37/4.78 and p41/4.75) were identified as tropomyosins, a group of at least five cytoskeletal proteins. Transformation by the papovaviruses simian virus 40 and polyomavirus caused no suppression of synthesis of these tropomyosins. This indicates that suppression of tropomyosin synthesis is not a nonspecific response by cells to being forced to grow with the transformed phenotype but is specifically associated with oncogenesis by diverse retroviral oncogenes. The results are consistent with the hypothesis that the different biochemical processes initiated by expression of structurally diverse retroviral oncogenes may converge on a limited number of common targets, one of which is the mechanism which regulates the synthesis of tropomyosins.

The genomes of many oncogenic retroviruses contain one or more genetic elements (oncogenes) that encode protein products able to transform cells in vitro and to cause tumors in vivo. These retroviral oncogenes are derived from normal cellular sequences (protooncogenes) by recombinational events not yet clearly understood (4, 5, 15, 39). Oncogenes related to normal cellular sequences have also been isolated from spontaneous and induced tumors in several animal species, including humans, by DNA-mediated transfection techniques (14, 19, 35, 38). Molecular studies have shown that the oncogenes present in different retroviruses as well as those isolated from individual tumors or from transformed cell lines are often structurally related or identical to one another, suggesting that the number of different cellular sequences which can serve as protooncogenes is limited.

The mechanism by which the protein products of oncogenes cause neoplastic cell transformation is not understood. It is therefore not possible to assess completely the functional relationships among oncogenes which exhibit different structures, although a few apparent functional similarities among structurally distinct oncogenes have already been noted (23, 32). The ultimate effects of oncogene expression, however, are often quite similar, even in cases for which little or no structural relationship has been demonstrated among the oncogenes themselves. Thus, in culture systems such as continuous mouse 3T3 cell lines, introduction of structurally dissimilar oncogenes may often be associated with strikingly similar effects on host cells, including characteristic changes in morphology, loss of contact inhibition of growth, replication in suspension cultures, and tumor formation in susceptible animals.

One possible explanation for this observation is that distinct oncogene products may initially affect different biochemical processes which are essential to and converge on a limited number of final common pathways whose functions are required for maintenance of the normal cell growth pattern. An agent whose presence ultimately alters these common pathways may be oncogenic, regardless of its initial site of action. To elucidate the mechanisms of neoplastic transformation fully it is necessary to identify and study possible common pathways for oncogenesis in addition to searching for the initial targets of individual oncogene products.

One approach to this question is exemplified by two recent studies in which total cell proteins synthesized by control cells were compared with those of transformed cell lines by two-dimensional polyacrylamide gel electrophoresis (6, 18). In these studies the oncogenic virus was not a retrovirus, but was simian virus 40 (SV-40), a DNA tumor virus whose transforming genes may differ from those of the retroviruses. As is often the case in electrophoretic studies of this type, in which hundreds of newly synthesized proteins are visualized, large numbers of quantitative differences in the synthesis of specific proteins were observed. The most suggestive finding was that the great majority of changes involved a reduction in synthesis of specific proteins in transformed cells rather than the appearance or induction of new proteins.

A major concern when studying transformed cell lines by two-dimensional analysis of cell protein complements arises from the fact that the cells employed are clones derived from

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cell culture lines and are generally aneuploid. Therefore, it is difficult to determine which of the many observed differences in protein synthesis are related to transformation and which are the result of selection in culture or of other in vitro effects. In searching for specific proteins whose synthesis may be altered as common pathway events in oncogenesis, we attempted to overcome this problem by comparing two-dimensional gel patterns of proteins from NIH/3T3 cells with those of a number of independently produced cloned lines of NIH/3T3 transformed by v-*ras*^{Ki}, the oncogene of Kirsten murine sarcoma virus. Modifications which appeared to be transformation related (rather than clone specific) were then examined in clones transformed by the closely related v-*ras*^{Ha} oncogene as well as in clones transformed by four other retroviral oncogenes. Changes which were found to be common among all lines were assessed in a line of cells in which expression of the v-*ras*^{Ha} oncogene as well as morphological alteration are corticosteroid dependent and reversible (22; N. Nikhat-Najam, M. Noda, I. Unnisa-Ali, G. L. Hagar, and R. H. Bassin, manuscript in preparation). Finally, we examined two recently described cellular revertant lines in which a v-*ras*^{Ki}-transformed NIH/3T3 cell line resumed contact-inhibited growth and lost tumorigenicity, despite retention of the oncogene and continued synthesis of the oncogene product, p21 (32).

These comparative studies allowed us to identify a small number of proteins which might be final common targets of structurally diverse retroviral oncogenes. Prominent among them was a pair of proteins which we identified as tropomyosins, which have been previously shown to be suppressed during oncogenesis in other systems (20, 21, 26, 29, 30).

MATERIALS AND METHODS

Cell lines. In this study, all cell lines transformed by retroviral oncogenes were clonal derivatives of NIH/3T3 cells either infected with a defective transforming retrovirus in the absence of replicating helper virus or transfected with the appropriate DNA sequence. Cells transformed by v-*ras*^{Ki} (see reference 10 for nomenclature of retroviruses) included K-NIH (gift of E. M. Scolnick), DT (32), and clones IC^{3}7, IC^{3}8, IC^{3}9, and ID^{3}2 (isolated in our laboratory). All of these lines were derived from cells infected with Kirsten murine sarcoma virus 3. Lines 551 and 568 were derived from NIH/3T3 cells transfected with v-*ras*^{Ha} (gift of D. Lowy). Line SL924 was derived from Moloney murine sarcoma virus (v-*mos*)-infected cells (gift of A. Reis). The GHN line of NIH/3T3 cells transfected with v-*fes*, and the 2-1 line of cells transfected with v-*src* have been described previously (1, 16). The 504-transformed line (v-*fms*) was provided by C. J. Sherr. The SV-3T3 cell line derived from Swiss 3T3 cells transformed by the papovavirus SV-40 and the polyomavirus-transformed cell line Py6-JM were obtained from M. Israel. Cells were cultivated in Dulbecco minimal essential medium supplemented with 10% fetal bovine serum and antibiotics.

Radiolabeling. Unless otherwise noted, cells were radiolabeled at 50 to 70% confluence. Monolayers in T75 flasks were rinsed once with phosphate-buffered saline (PBS), and 8 ml of leucine-free medium RPMI 1640 supplemented with 5% dialyzed fetal bovine serum and [^{3}H]leucine (50 μCi/ml, 130 Ci/mmol; Amersham Corp.) was added. After incubation for 4 h, labeling medium was removed, and cells were rinsed once with PBS and scraped from the flask with a rubber policeman into 10 ml of cold PBS. Cells were recovered by centrifugation (600 × *g*).

Two-dimensional polyacrylamide gel electrophoresis. Cells were lysed by vortexing for 10 s in 100 to 200 μl isoelectric focusing (IEF) lysis solution (9.5 M urea, 2% Nonidet-P40, 5% 2-mercaptoethanol, 2% ampholytes [pH 3.5 to 10 LKB Instruments]). Nuclear residue was removed by centrifugation. Volumes of lysate containing 2 × 10^6 cpm of acid-insoluble radioactivity were analyzed by two-dimensional electrophoresis as described previously (13), using the Anderson apparatus (Electronucleonics). All first-dimension gels contained 2% ampholytes, pI 3.5 to 10. For proteins with M_rs up to 200,000, second-dimension gels were 7.5% polyacrylamide. For proteins with M_rs below 100,000, 12% second-dimension gels were used. The pH gradient of isoelectrically focused first-dimension gels was determined from extracts in boiled, distilled water of segments of blank gels. Focused gels were run for 9,000 to 10,000 voltage hours, with the sample loaded at the basic end. Nonequilibrium pH gradient electrophoresis gels were run for 5,000 to 6,000 voltage hours, with the sample loaded at the acidic end. Because proteins, especially those with higher M_rs, may not reach their isoelectric position during nonequilibrium pH gradient electrophoresis runs, no attempt was made to estimate pI values in those cases. Gels were stained with Coomassie blue and processed for fluorography as described previously (12) or were stained by the silver technique of Morrissey (31).

Preparation of CSK and SOL fractions. Monolayers of radiolabeled cells were prepared in six-well culture plates (Falcon; 35-mm wells). After being rinsed twice with PBS, 0.4 ml of cytoskeletal (CSK) extraction buffer (0.1 M PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] [pH 6.9], 5 mM $MgCl_2$, 0.2 M EGTA, 0.05% Triton X-100, 0.1 mM phenylmethylsulfonyl fluoride, 4 M glycerol) was added to each well. After 2 min at room temperature, the plate was rocked gently, the extraction buffer was collected by aspiration, and proteins were precipitated from it by addition of 5 volumes of cold acetone-NH_4OH (17:1). Precipitated detergent-soluble (SOL) proteins were recovered by centrifugation and dissolved in IEF lysis solution. Detergent-insoluble structures adherent to the culture plate were rinsed three times with PBS and then scraped up in 0.3 ml of IEF lysis buffer, transferring the same portion of IEF lysis buffer to successive culture wells. After being vortexed for 10 s, nuclear residues were removed by centrifugation and the supernatant recovered as the CSK fraction.

Immunoprecipitation of tropomyosins. Radiolabeled cells were lysed by being vortexed for 10 s after suspension in 0.3 to 0.5 ml of disruption buffer (0.01 M NaCl, 0.01 M Tris-chloride [pH 7.4], 1% Nonidet P-40, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride). Nuclei were removed by centrifugation. Salt concentration was adjusted to 0.15 M NaCl, and pH was adjusted to ca. 8.3 by the addition of 1.0 M Tris-chloride (pH 8.8). Cytoplasmic lysates (300 μl) were incubated twice for 30 min with the resuspended pellet of 100 μl of a 10% suspension of Formalin-fixed, heat-killed staphylococcus A (Pansorbin; Calbiochem-Behring). Staphylococci were removed by centrifugation (10,000 × *g*, 3 min) and 20 μl of rabbit antiserum to chicken muscle tropomyosin (kindly provided by F. Matsumura) was added. Normal rabbit serum was used in a control preparation. After 30 min of incubation at room temperature, the 100-μl pellet of Pansorbin was suspended in the lysate. After an additional 30-min incubation, the pellet was recovered by centrifugation and washed once with disruption buffer and again with disruption buffer containing 0.1% sodium dodecyl sulfate in place of Nonidet P-40. The staphylococcal pellet was sus-

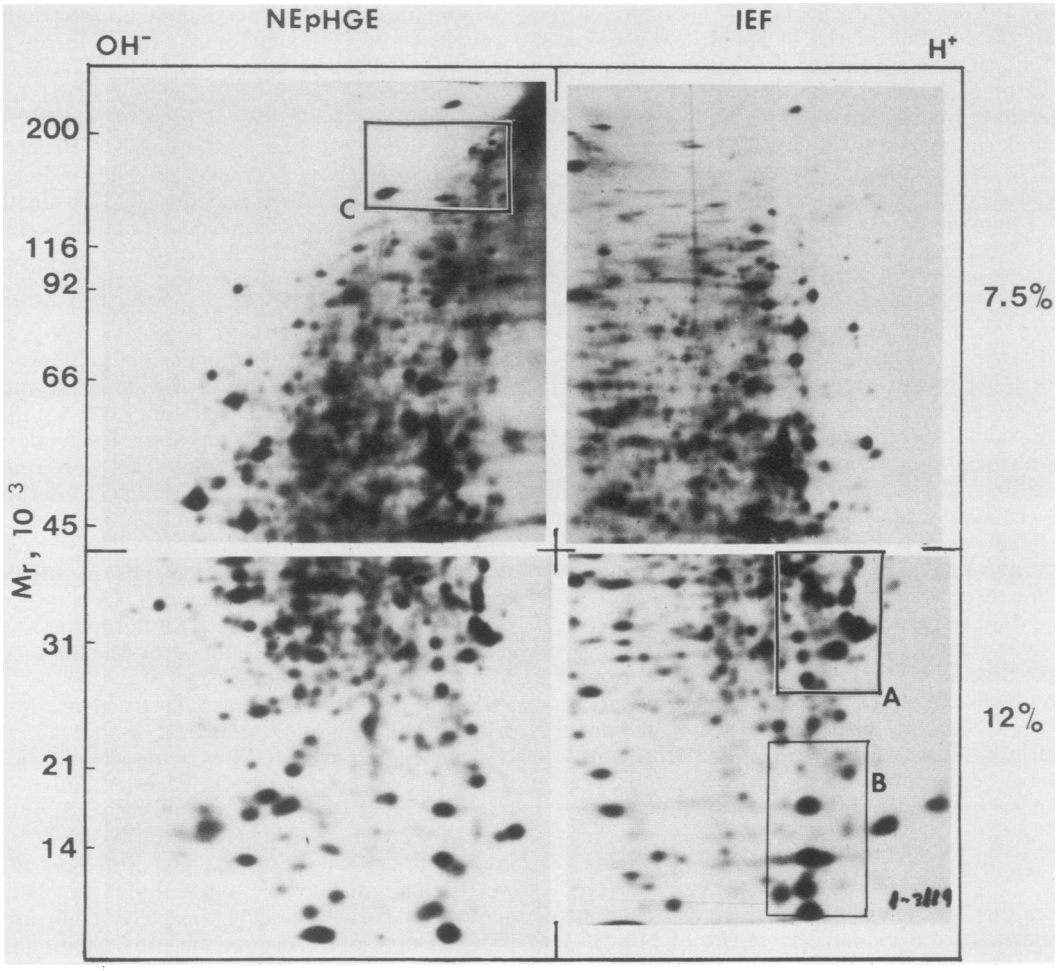

FIG. 1. Newly synthesized proteins of NIH/3T3 cells analyzed by two-dimensional polyacrylamide gel electrophoresis. Subconfluent monolayers of NIH/3T3 cells were labeled for 4 h with [^{3}H]leucine. Whole cell proteins were prepared, analyzed by two-dimensional polyacrylamide gel electrophoresis, and visualized by fluorography as described in the text. Patterns are displayed with acidic proteins to the right. Proteins with pIs in the range 3.5 to 7.2 were separated in the first dimension by isoelectric focusing (right-hand panels). Proteins with more basic pIs were separated in the first dimension by nonequilibrium pH gradient electrophoresis technique, using a 6,000-voltage hour running time (left-hand panels). In this display, proteins with M_rs of less than or equal to 45,000 were separated on 7.5% uniform concentration polyacrylamide gels in the second dimension (upper panels), and smaller proteins were separated on 12% gels (lower panels). In general, proteins as large as 100 kDa could also be analyzed on 12% gels (see Fig. 2). Areas outlined (A, B, and C) contain proteins of special interest which are illustrated in Fig. 3 and 4.

pended in 50 μl of IEF lysis solution and incubated for 1 h at 37°C. After removal of the staphylococci by centrifugation, the recovered proteins were mixed with 15 μl of a 1:20 dilution in IEF lysis solution of Bio-Rad low-molecular-weight electrophoresis protein standards, and the entire sample was analyzed by two-dimensional electrophoresis. A sample of the original cytoplasmic lysate was analyzed after acetone precipitation.

Heat-resistant proteins. Cytoplasmic lysate was prepared by nitrogen cavitation as described before (36). After heating at 100°C for 10 min, precipitated proteins were removed by centrifugation for 10 min at 10,000 × g. Proteins remaining in the supernatant were recovered by acetone precipitation as above. Control lysates were treated identically except for omission of heating.

RESULTS

Comparison of NIH/3T3 and DT lines. Newly synthesized proteins in NIH/3T3 cells and in the DT clone of NIH/3T3 cells transformed with v-*ras*^{Ki} were radiolabeled with [^{3}H]leucine. Whole-cell proteins were analyzed by two-dimensional polyacrylamide gel electrophoresis, as described above, to permit examination of proteins of 10,000 to 200,000 in the M_r dimension and acidic as well as basic proteins in the pI dimension. A radiofluorogram of the proteins of NIH/3T3 cells is shown in Fig. 1. Similar analyses were made of all the cell lines studied. Equivalent amounts of radioactivity were analyzed in all cases.

Comparison of fluorograms of two-dimensional displays of DT and NIH/3T3 proteins was performed visually. Synthesis of a protein was considered to be significantly decreased in DT cells if the labeling intensity of that protein was greater than that of neighboring proteins in control NIH/3T3 cells but less than those same neighboring proteins in DT cells. Increased synthesis of a protein in DT cells was defined by the opposite relationship. Such reversal of relative labeling intensity was required to be evident in three separate comparisons. We have previously shown that changes scored in this way always indicated differences in actual radioactivity of greater than or equal to twofold (12).

TABLE 1. Modified synthesis of 27 cellular proteins in the DT cell line compared with NIH/3T3 cells

M_rpI[a]	Direction[b] of change
1. 200/basic	Decrease
2. 175/basic	Decrease
3. 87/6.1	Decrease
4. 57/6.5	Increase
5. 56/5.78	Decrease
6. 56/5.65	Decrease
7. 52/5.72	Decrease
8. 52/4.77	Decrease
9. 52/4.65	Increase
10. 48/4.65	Decrease
11. 48/4.6	Decrease
12. 41/4.75	Decrease
13. 38/4.78	Decrease
14. 37/4.78	Decrease
15. 37/6.76	Increase
16. 37/5.6	Decrease
17. 36/5.85	Decrease
18. 33/5.3	Decrease
19. 32/5.83	Decrease
20. 32/5.7	Decrease
21. 27/5.8	Decrease
22. 25/5.4	Decrease
23. 21/4.8	Decrease
24. 21/7.2	Decrease
25. 19/5.8	Decrease
26. 13.4/5.4	Increase
27. 11/5.4	Decrease

[a] pI for proteins visualized only on nonequilibrium pH gradient electrophoresis gels is given only as basic since estimates of pI from such gels is highly inaccurate especially for higher-molecular-weight proteins.
[b] Labeling intensity in DT relative to that in NIH/3T3.

Initially, 27 proteins were selected for further consideration based on evident differences in their synthetic rates between NIH/3T3 and DT cell preparations. Each of these proteins is listed in Table 1, together with its M_r, approximate pI, and direction of change. Other quantitative changes in labeling were noted but were not included for further study because of questionable conformity to the scoring described above. The location of the selected proteins in the electropherogram is indicated in Fig. 2 for all but the two proteins of highest molecular weight. The latter proteins are seen in enclosed area C (Fig. 1 and 4). The numbers in Fig. 2 are provided for convenience of reference to Table 1. However, specific proteins will always be designated by M_r/pI in the text.

The level of sensitivity of this type of analysis probably allows detection of no more than the 15% most prominently synthesized proteins (27). In this report, then, we deal only with proteins which are relatively abundant cell components. The potential importance of quantitatively minor constituents remains to be determined.

All but four of the selected proteins showed diminished synthesis in DT cells (Table 1). This observation agrees with previous reports of the predominance of reductions in synthesis of specific proteins in virally transformed cells (6, 18). Examples of the more prominent changes are illustrated in Fig. 3 and 4, in which the areas enclosed in rectangles in Fig. 1 are shown for 3T3 and DT cells as well as for the revertant cell lines to be discussed. The proteins delineated by rectangle A in Fig. 1 are shown in Fig. 3. Synthesis of three [^{3}H]leucine-labeled proteins (37, 38, and 41 kilodaltons [kDa]) is markedly suppressed in DT as compared with NIH/3T3 cells. Areas B and C of Fig. 1 are shown in Fig. 4. Suppression of synthesis of proteins of 11, 21, 175, and 200 kDa was observed in DT cells, whereas a protein at 13 kDa was one of the few showing enhanced synthesis.

Examination of other cell lines transformed by v-*ras*^{Ki}. If alteration in synthesis of a given protein is part of the mechanism by which a particular oncogene causes transformation, then other cell lines transformed by that oncogene should exhibit the same changes. To study this point, five other cloned lines of NIH/3T3 cells transformed by v-*ras*^{Ki} were examined and scored for relative synthesis of the 27 proteins listed in Table 1. Agreement or disagreement with the findings in DT for each protein is shown in Table 2. Where synthesis of a given protein was similarly changed in all six v-*ras*^{Ki}-transformed lines, the protein has been footnoted.

This comparison showed that 10 proteins of the original group of 27 were altered consistently and concordantly in lines transformed by v-*ras*^{Ki}. Significantly, this group in-

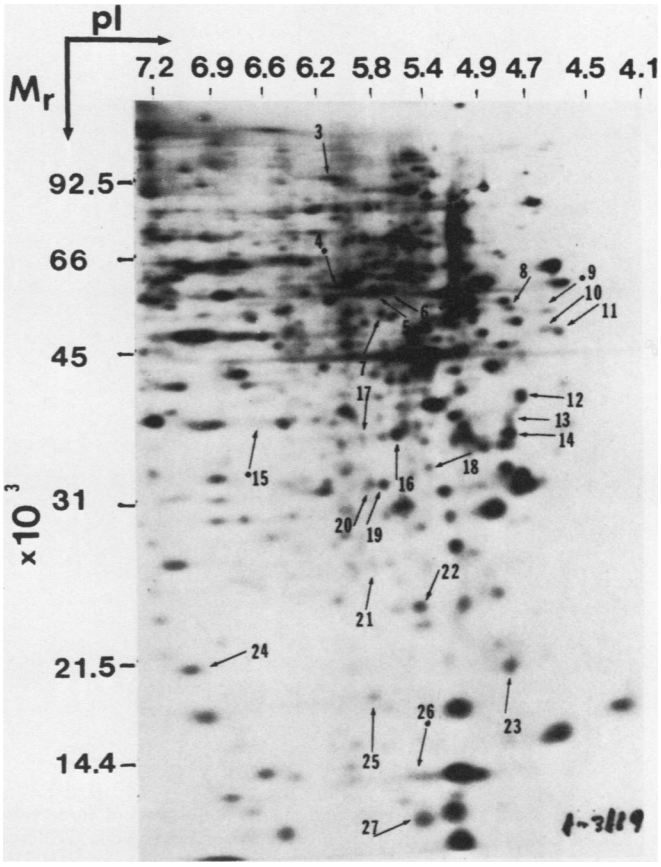

FIG. 2. Proteins whose synthesis was modified in the DT cell line transformed by the v-*ras*^{Ki} oncogene. Newly synthesized proteins of NIH/3T3 cells analyzed by two-dimensional electrophoresis are displayed. A portion of this fluorogram was used for the lower right panel of Fig. 1. Proteins numbered 3 to 27 in Table 1 are indicated by arrows. Numbers are assigned to proteins in this figure to facilitate comparison with Table 1. All other references to specific proteins are in the form M_r/pI. High-molecular-weight, basic proteins numbered 1 and 2 in Table 1 were not visualized under these conditions but are seen in the area designated C in Fig. 1 and in Fig. 4. Arrows with terminal dots indicate proteins whose synthesis was relatively increased in DT cells. All other indicated proteins showed diminished synthesis in the DT cell line.

cluded only proteins showing reduced synthesis in transformed cells. It is likely that the four proteins whose synthesis was enhanced in DT cells as well as the other 13 proteins exhibiting reductions in synthesis which were not consistent among v-*ras*^{Ki} transformants had undergone modifications in regulation of their synthesis in particular cell lines independently of the transformation process. Interestingly, several of the rejected proteins listed in Table 2 showed changes concordant with DT cells in four of five cases. This suggests that selection pressure may exist which favors recurrence of particular transformation-independent changes. This observation underscores the need for care in interpretation of changes in synthesis of specific proteins in cloned cell lines. Although it is possible that recurrent changes are transformation related, and that the cases lacking such changes are the result of complex alterations rendering them unnecessary, it seems unsafe to make that conclusion in the absence of further information on the function of the proteins involved.

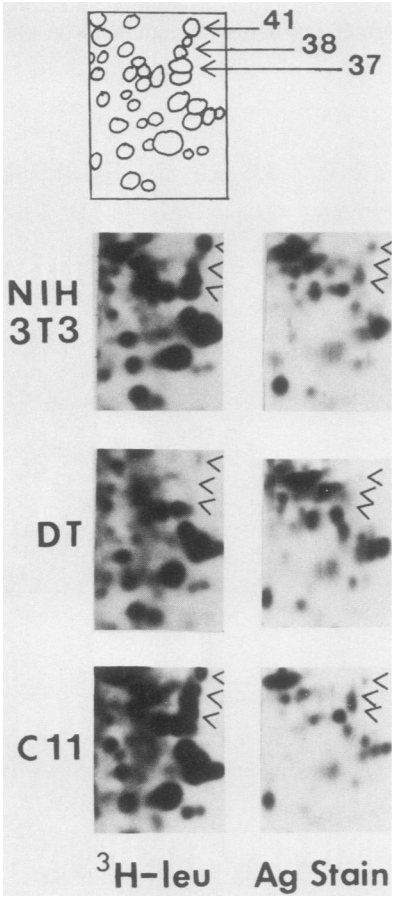

FIG. 3. Modified synthesis and steady-state level of three proteins in control, transformed, and revertant NIH/3T3 cells. Proteins located in area A indicated in Fig. 1 are shown. Proteins of NIH/3T3 cells, DT cells (v-*ras*^{Ki}-transformed), and C-11 cells (cellular revertant of DT) were radiolabeled for 4 h with [^{3}H]leucine. Whole cell proteins were analyzed by two-dimensional electrophoresis in duplicate. One member of each gel pair was prepared for fluorography to visualize newly synthesized proteins; the second gel was silver stained to estimate steady-state content of proteins. Left-hand panels are fluorographs and right-hand panels are Ag stained. Upper panels are NIH/3T3; middle panels are DT; and lower panels are C-11. Drawing at top identifies proteins of interest by M_r. The protein designated 38 is p38/4.78 of Table 1. Because of its generally poor resolution from p37/4.78 and generally light labeling intensity, judgments about it were considered uncertain. The other two proteins indicated are p37/4.78 and p41/4.75 of Table 1.

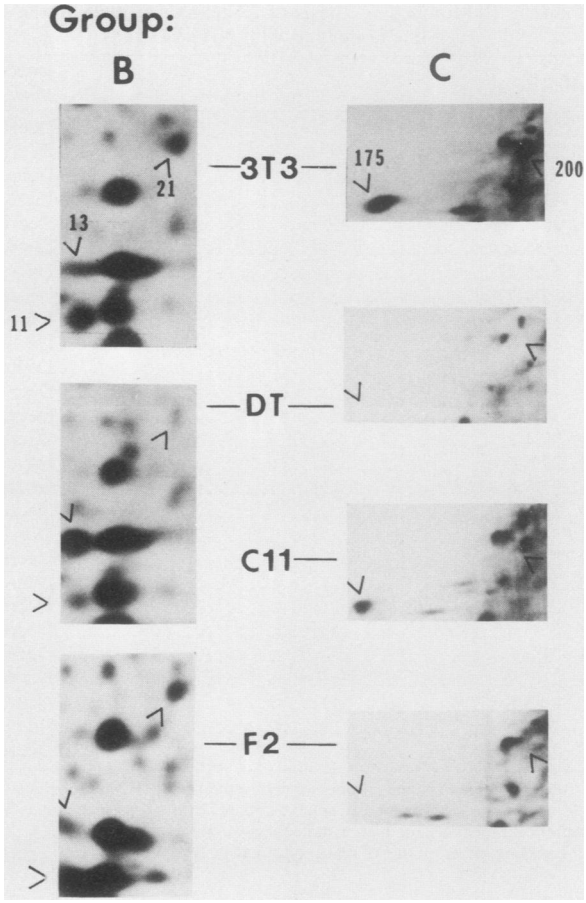

FIG. 4. Modified synthesis of group B and C proteins in control, transformed, and revertant cells. Fluorographs of newly synthesized proteins located in areas B and C of Fig. 1 are shown. Left-hand panels; group B proteins; right-hand panels; group C proteins. Cell line F-2, like C-11, is a cellular revertant of line DT. Proteins of interest are indicated by arrowheads and identified by M_r in upper panels, corresponding to M_r designations in Table 1.

Comparison of v-*ras*^{Ki} and v-*ras*^{Ha} transformants. The v-*ras*^{Ki} and v-*ras*^{Ha} oncogenes arose independently during passage in rats of Kirsten and Moloney murine leukemia viruses, respectively (for review see reference 39). They are probably derived from different closely related members of c-*ras* multigene family. The v-*ras*^{Ha} oncogene differs from the v-*ras*^{Ki} oncogene primarily in its -COOH terminal coding region but also at the critical codon 12 position. The *gly* codon of the normal (c-*ras*) gene at that position is replaced by a *ser* codon in v-*ras*^{Ki} but by an *arg* codon in v-*ras*^{Ha}. It was of interest, therefore, to determine whether these differences in amino acid sequence of the oncogene product influenced the synthesis of any of the proteins suppressed in v-*ras*^{Ki} transformants.

Newly synthesized proteins in two lines of v-*ras*^{Ha}-transformed cells (lines 568 and 551) were compared with the proteins of NIH/3T3 and DT lines by two-dimensional gel electrophoresis. Of the 10 proteins consistently suppressed in v-*ras*^{Ki} transformants, 9 were also clearly suppressed in both v-*ras*^{Ha} transformants (Table 3). The remaining protein,

TABLE 2. Concordance of changes in synthesis of 27 proteins in line DT[a] with changes in five other cell lines transformed by v-*ras*^{Ki}

Protein	Concordance in cell line[b]:				
	KNIH	IC^{3}7	IC^{3}8	IC^{3}9	ID^{3}2
200/basic[c]	+	+	+	+	+
175/basic[c]	+	+	+	+	+
87/6.1	+	+	+	+	−
57/6.5	−	−	−	+	−
56/5.78	−	−	+	−	−
56/5.65	−	−	+	−	−
52/5.72[c]	+	+	+	+	+
52/4.77	−	−	+	−	+
52/4.65	−	−	−	−	+
48/4.65	+	+	+	−	−
48/4.6[c]	+	+	+	+	+
41/4.75[c]	+	+	+	+	+
38/4.78[c]	+	+	+	+	+
37/4.78[c]	+	+	+	+	+
37/6.76	−	−	−	−	+
37/5.6[c]	+	+	+	+	+
36/5.85	+	+	−	+	+
33/5.3	−	−	+	−	−
32/5.83	−	+	+	+	+
32/5.7	±	±	+	+	+
27/5.8	+	+	−	+	−
25/5.4	+	+	+	+	−
21/4.8[c]	+	+	+	+	+
21/7.2[c]	+	+	+	+	+
19/5.8	±	+	±	+	±
13.4/5.4	−	−	−	−	−
11/5.4	−	−	−	−	−

[a] All listed proteins showed changes in synthesis in DT cells compared with NIH/3T3. See Table 1.

[b] +, Concordant. Change in synthesis compared with NIH/3T3 is similar to DT; −, nonconcordant. No change in synthesis compared with NIH/3T3; ±, uncertain. Observed changes were too slight to be scored visually.

[c] Concordant changes in all v-*ras*^{Ki}-transformed lines tested.

p38/4.78, was always faintly labeled and poorly resolved, and judgments about it cannot be considered reliable. It will not be considered further.

Cells transformed by retroviral oncogenes unrelated to *ras*. To determine whether suppression of synthesis of any of the nine proteins described above was specific for transformation by v-*ras* or might be a more general feature of transformation by retroviruses, synthesis of these proteins was examined in several cell lines transformed by oncogenes unrelated to v-*ras*. Clones of NIH/3T3 cells transformed by the *mos*, *fms*, *fes*, and *src* oncogenes were analyzed by two-dimensional electrophoresis of newly synthesized proteins. Table 3 shows that five of the nine proteins suppressed in v-*ras* transformants were also suppressed in all other transformed lines examined (footnoted).

Three other proteins must be noted, however. Proteins p37/5.6, p21/4.8, and p21/7.2 showed concordant suppression in all lines examined, with the exception of v-*src*-transformed line 2-1. Although these proteins may be further examples of recurrent changes arising during cultivation, it is also possible that the biochemical pathway leading to transformation by the *src* oncogene may differ in some respects from that used by the other retroviral oncogenes studied.

Corticosteroid-inducible cells. To minimize the possibility that reduced synthesis of any or all of the proteins described above might have resulted from random variation or selection or both among different transformed clones of the NIH/3T3 cell line, we studied a hormone-sensitive system described by Huang et al. (22). The 433.3 cell line is a clone of NIH/3T3 cells transfected with a molecular chimera composed of the v-*ras*^{Ha} oncogene covalently linked to the long-terminal repeat of mouse mammary tumor virus. Transcription of the v-*ras*^{Ha} gene in these cells is under control of the mouse mammary tumor virus promoter which functions only in the presence of glucocorticoid hormone. Synthesis of *ras*-specific RNA increases after the addition of dexamethasone to 433.3 cells, and new synthesis of p21 *ras* protein can be demonstrated within 4 h. Morphological changes in 433.3 cells and increased colony formation in semisolid agar are also dependent upon the presence of glucocorticoids and are most evident when the cells are grown under serum-free conditions, in which changes in the level of p21 synthesis after the addition or removal of glucocorticoids are most dramatic (Nikhat-Najam et al., in preparation). Since these changes occur soon after the addition of glucocorticoids without passage of the cells, the possibility of selective overgrowth of cells with specific characteristics unrelated to the induction of oncogene expression is minimized.

Duplicate plates of 433.3 cells were incubated in serum-free medium with and without the addition of dexamethasone (10^{-7} M). At daily intervals, control and treated plates were radiolabeled for 4 h and newly synthesized proteins were examined by two-dimensional electrophoresis. The electropherogram of newly synthesized proteins in control 433.3 cells was essentially identical to that of NIH/3T3 cells (data not shown). After 4 days of exposure to dexamethasone, 433.3 cell cultures showed a degree of morphological alteration ranging from about 50% to nearly complete in different experiments. Of the eight proteins that were generally suppressed in cloned, transformed cells (with the exception of 2-1 cells), only two (p37/4.78 and p41/4.75) were also suppressed after virtually complete hormone-induced morphological alteration of 433.3 cells (Table 4, footnote *b*). These proteins were localized to region A, indicated in Fig. 1. That region from control 433.3 cells and from cells after 4 days of corticosteroid exposure is shown in Fig. 5. The morphological appearance of the cells used for the experiment shown in Fig. 5, after 4 days of exposure to dexamethasone, was indistinguishable from that of the transformed DT cell line. The reduction in relative labeling of p37/4.78 and

TABLE 3. Concordance of changes in synthesis of 10 proteins suppressed in v-*ras*^{Ki}-transformed lines with changes in cell lines transformed by other oncogenes

Protein[a]	Concordance in cell line and oncogene transformation[b]:					
	568, v-*ras*^{Ha}	551, v-*ras*^{Ha}	SL924, *mos*	*504*, *fms*	GHN, *fes*	2-1, *src*
200/basic[c]	+	+	+	+	+	+
175/basic[c]	+	+	+	+	+	+
52/5.72	+	+	+	−	−	−
48/4.6[c]	+	+	+	+	+	+
41/4.75[c]	+	+	+	+	+	+
38/4.78	−	+	−	+	+	+
37/4.78[c]	+	+	+	+	+	+
37/5.6	+	+	+	+	+	−
21/4.8	+	+	+	+	+	−
21/7.2	+	+	+	+	+	−

[a] The 10 starred proteins shown in Table 2 are listed.

[b] +, Concordant. Change in synthesis compared with NIH/3T3 is similar to that in DT; −, nonconcordant. No change in synthesis compared with NIH/3T3.

[c] Concordant changes in all lines tested.

p41/4.75 was clearly evident by day 2, although only occasional areas of the culture exhibited morphological alteration at that time (data not shown). Other changes apparent in Fig. 5 were not consistently found and may be associated with glucocorticoid treatment rather than with oncogene expression.

Thus, p37/4.78 and p41/4.75 emerge as the only proteins whose synthesis is consistently suppressed not only in lines transformed by each of the retroviral oncogenes we have studied, but also during acquisition of the transformed phenotype under conditions which minimize selection. This does not exclude the possibility that alterations in synthesis of other proteins listed in Table 4 may be required for prolonged maintenance of transformed growth beyond the initial induction period.

Revertant cell lines. The availability of cellular revertants derived from DT cells by mutagen treatment and selection for the nontransformed phenotype in the presence of ouabain (32) makes possible a critical test of the relationship of suppressed synthesis of specific proteins to the transformation process. These cells (lines C-11 and F-2) retain the v-*ras*^{Ki} oncogene and have been reported to synthesize the transforming gene product, p21 *ras*, in amounts greater than control NIH/3T3 cells. They also continue to elaborate a soluble tumor growth factor. However, the revertants do not exhibit the spindle-shaped or rounded cell morphology of the transformed DT line. They express contact inhibition of growth, do not form colonies in semisolid medium, and are nontumorigenic in nude mice (32). If continued synthesis of any or all members of the set of eight proteins described above is essential for maintenance of the nontransformed growth pattern, then synthesis of those proteins should be restored in cells reverting to the nontransformed phenotype.

Newly synthesized proteins of the C-11 and F-2 revertant lines were compared with those of NIH/3T3 cells and with those of the parent DT line from which they were derived. Of the eight proteins whose synthesis was suppressed in DT and in all other lines transformed by retroviral oncogenes other than *src*, five showed restored synthesis in both revertant lines (Table 4, footnote *c*). These included p37/4.78 and p41/4.75, which were also the only proteins suppressed during glucocorticoid-induced transformation of 433.3 cells and were suppressed by *src* as well. Figure 3 shows regions of fluorographs containing p37/4.78 and p41/4.75, illustrating their strong suppression in DT and restoration in the revertant C-11. The revertant F-2 showed identical behavior (data not shown). The remaining proteins showing restoration in both revertants were p48/4.6, p37/5.6, and p21/4.8, whereas p200/basic and p175/basic were both restored in only one revertant line (Table 4).

TABLE 4. Synthesis of eight proteins during induction of transformation in a corticosteroid-sensitive cell line and in revertant cell lines

Protein[a]	Suppression in line 433.3[b]	Restored in revertant line:	
		C-11	F-2
200/basic	N	Y	N
175/basic	N	Y	N
48/4.6[c]	N	Y	Y
41/4.75[c,d]	Y	Y	Y
37/4.78[c,d]	Y	Y	Y
37/5.6[c]	N	Y	Y
21/4.8[c]	N	Y	Y
21/7.2	N	N	N

[a] The proteins listed in Table 3 are listed here, with the exception of p52/5.72 and p38/4.78 (see the text).

[b] Y, Yes; N, no.

[c] Restored toward control level synthesis in both revertant lines, C-11 and F-2.

[d] Suppressed during corticosteroid induction of v-*ras*^{Ha} expression in line 433.3.

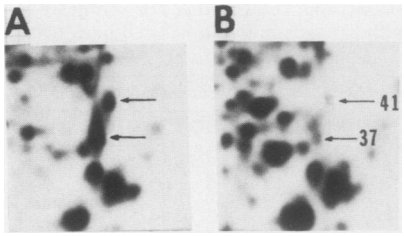

FIG. 5. Suppression of synthesis of p37/4.78 and p41/4.75 during induction of v-*ras*^{Ha} expression by corticosteroids. Cells of line 433.3 were cultured in serum-free medium with or without addition of 10^{-7} M dexamethasone. Cells were then labeled for 4 h with [^{3}H]leucine, and newly synthesized proteins were analyzed by two-dimensional electrophoresis. (A) Control 433.3 cells. (B) 4 days of incubation with dexamethasone. Arrows indicate locations of p37/4.78 and p41/4.75. In the experiment illustrated, corticosteroid-treated cells showed virtually 100% morphological alteration at the time of labeling.

The steady-state content of p37/4.78 and p41/4.75 was examined by silver staining of two-dimensional gels (Fig. 3). Again, marked reduction was evident in DT cells, whereas C-11 cells (and F-2 cells [data not shown]) showed no apparent diminution in their content. Evidently the diminished synthetic rate of proteins in this group results in a marked reduction in their quantity in DT cells, while reversion restores their content.

Thus, of the eight proteins whose synthesis was generally suppressed in established cell lines transformed by retroviral oncogenes, one is apparently not essential for restoration of the nontransformed phenotype in transformed cells (p21/7.2). Of the remaining seven proteins, modifications in synthesis of p37/4.78 and p41/4.75 were associated with both induction of morphological alteration and oncogene expression in hormone-responsive cells and with restoration of the nontransformed phenotype in revertant lines. Proteins p48/4.6, p37/5.6, and p21/4.8 were not suppressed during the corticosteroid-induced onset of morphological changes. However, their consistent suppression in established lines of transformed cells together with their restoration in both revertants suggests that reduced synthesis of these proteins may be involved in maintaining the transformed state in culture and that restoration of their synthesis, as well as that of p37/4.78 and p41/4.75, may be necessary to permit the reappearance of the nontransformed phenotype in revertants. The same may be true of p200/basic and p175/basic, although the failure of restoration of their synthesis in one revertant line suggests that their function may not be absolutely required for expression of the nontransformed phenotype.

Subcellular localization of suppressed proteins. The level of production of p37/4.78 and p41/4.75 is seen to be closely correlated with expression of the transformed or nontransformed cell growth pattern, making these proteins excellent candidates for final common targets of direct or indirect action by oncogene products. For further characterization, radiolabeled NIH/3T3 cells were extracted with low concentrations of nonionic detergent to fractionate their cytoplasm into SOL and CSK components (7, 9). Proteins of these fractions were analyzed by two-dimensional electrophoresis.

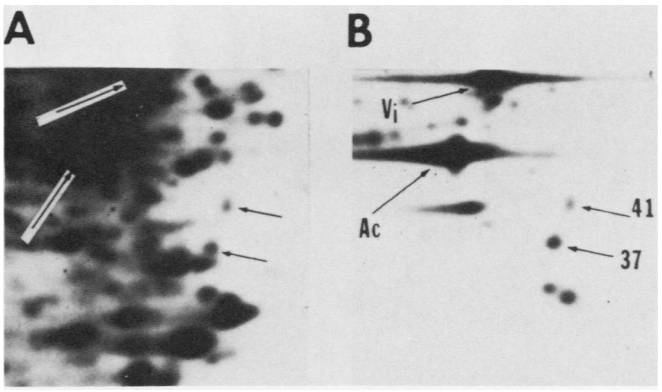

FIG. 6. Subcellular localization of p37/4.78 and p41/4.75. Distribution between SOL and CSK compartments. NIH/3T3 cells were radiolabeled for 4 h with [^{3}H]leucine and then extracted with a low concentration of nonionic detergent (see the text). The SOL and insoluble fractions were each analyzed by two-dimensional electrophoresis. (A) SOL proteins, overexposed fluorograph to detect p37/4.78 and p41/4.75. (B) CSK proteins. Locations of p37/4.78 and p41/4.75 are indicated by arrows. Also indicated are the CSK proteins actin (Ac) and vimentin (Vi).

p37/4.78 and p41/4.75 were well-represented among the proteins that remained with the detergent-insoluble structure, together with known CSK components (actin and vimentin) (Fig. 6B). In heavily exposed fluorographs of SOL proteins (Fig. 6A), p37/4.78 and p41/4.75 were also visible but were obviously minor components. Such distribution of CSK proteins between the CSK and SOL compartments has been noted previously (25, 41). These results suggest that p37/4.78 and p41/4.75 are CSK proteins.

Among the remaining proteins under consideration, p200/basic, p175/basic, and p21/4.8 were also detected in both CSK and SOL fractions, suggesting a relationship with the CSK, whereas p48/4.6 and p37/5.6 were detected only in the SOL fraction (data not shown). It is of considerable interest that, of the seven proteins that were consistently suppressed in cell lines transformed by retroviral oncogenes and fully or partially restored in revertants, five showed an association with the CSK.

Identification of p41/4.75 and p37/4.78 as tropomyosins. The molecular weight and pI values estimated for p37/4.78 and p41/4.75, together with their association with detergent-insoluble structures, correspond with reported characteristics of tropomyosins (20, 21, 26, 29, 30). We confirmed this possibility on the basis of (i) cross-reactivity with polyclonal rabbit antiserum to chicken gizzard tropomyosin (kindly supplied by F. Matsumura), (ii) the absence of the amino acid tryptophan from this group of proteins (40), (iii) the known heat stability of tropomyosins (2), and (iv) comparison with the two-dimensional electrophoretic mobility of purified rabbit muscle tropomyosin.

Cytoplasm was prepared from radiolabeled NIH/3T3 cells by nitrogen cavitation. Rabbit antiserum to chicken gizzard tropomyosin specifically bound to peptides in this extract which migrated identically to p37/4.78 and p41/4.75 (Fig. 7). Only minute amounts of total cell protein and no detectable p37/4.78 or p41/4.75 were recovered with control rabbit serum (data not shown).

NIH/3T3 cells were labeled with either [^{3}H]leucine or [^{3}H]tryptophan, and newly synthesized proteins were analyzed by two-dimensional electrophoresis. Neither p37/4.78 nor p41/4.75 was radiolabeled by [^{3}H]tryptophan (Fig. 8), confirming the absence of that amino acid from those proteins. Other apparent tryptophan-deficient proteins migrating in proximity to p37/4.78 and p41/4.75 are indicated in Fig. 8B. These proteins were evident in CSK fractions (Fig. 6) and may also be tropomyosins, in agreement with the results of Matsumura et al. (29, 30). However, these additional proteins did not show the strong cross-reactivity with the rabbit antiserum to chicken tropomyosin shown by p37/4.78 and p41/4.75 (Fig. 7). This result is also in agreement with the findings of Matsumura et al. (29, 30) with this antiserum.

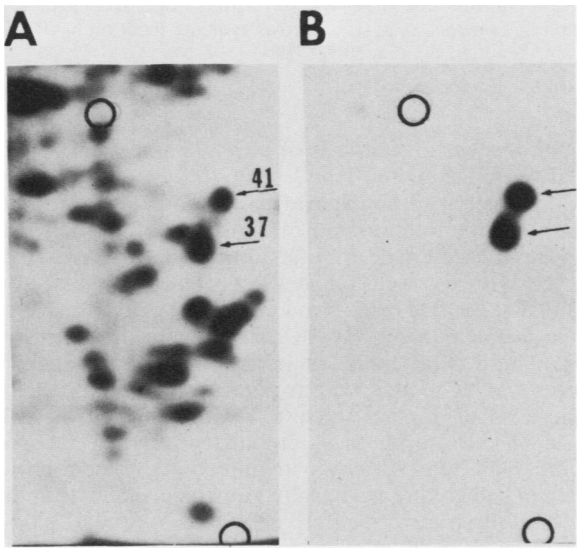

FIG. 7. Recognition of p37/4.78 and p41/4.75 by rabbit antiserum to chicken muscle tropomyosin. NIH/3T3 cells were radiolabeled for 4 h with [^{3}H]leucine. A detergent lysate of cytoplasm was prepared and proteins recognized by rabbit antiserum to chicken tropomyosin were recovered (see the text) and analyzed by two-dimensional electrophoresis and fluorography. (A) Untreated proteins of detergent lysate. (B) Proteins recognized by anti-tropomyosin antiserum. A control preparation with normal rabbit serum revealed no labeled proteins under the conditions employed (data not shown). Arrows indicate locations of p37/4.78 and p41/4.75. Drawn circles indicate locations of marker proteins ovalbumin and soybean trypsin inhibitor (45 and 21.5 kDa, respectively) mixed with samples and located by Coomassie blue staining to facilitate registration of the two fluorographs.

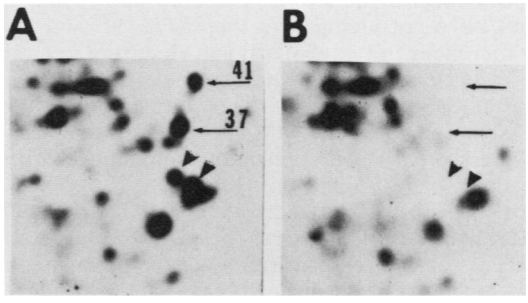

FIG. 8. Absence of tryptophan from p37/4.78 and p41/4.75. NIH/3T3 cells were radiolabeled for 4 h with either [^{3}H]leucine or [^{3}H]tryptophan. Whole cell proteins were analyzed by two-dimensional electrophoresis and fluorography. (A) [^{3}H]leucine label. (B) [^{3}H]tryptophan label. Arrows indicate locations of p37/4.78 and p41/4.75. Arrowheads indicate two other proteins migrating in close proximity to p37/4.78 and p41/4.75 which failed to label with [^{3}H]tryptophan.

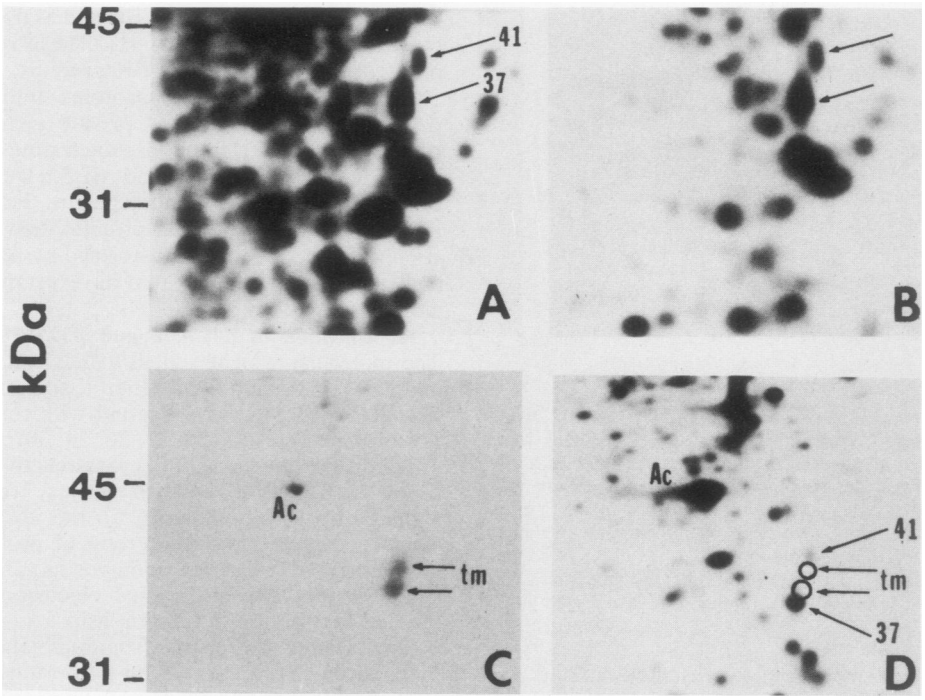

FIG. 9. Heat stability of p37/4.78 and p41/4.75 and comparison of two-dimensional electrophoretic mobility with purified rabbit muscle tropomyosin. (A) and (B) NIH/3T3 cells were radiolabeled for 4 h with [^{3}H]leucine and cytoplasm was prepared by N_2 cavitation. Heat-sensitive proteins were removed from a portion of the lysate (see the text) and remaining proteins were analyzed by two-dimensional electrophoresis and fluorography. (A) Proteins of unheated cytoplasm. (B) Heat-resistant proteins. (C) and (D) NIH/3T3 cells were radiolabeled for 4 h with [^{3}H]leucine. Whole cell proteins were prepared and mixed with 2 μg of purified rabbit muscle tropomyosin. The mixture was analyzed by two-dimensional electrophoresis, stained with Coomassie blue, and fluorographed. (C) Coomassie blue-stained gel. Position of added tropomyosin is indicated by tm. These spots were absent in a control stained gel without added tropomyosin (data not shown). Ac; Actin, derived from NIH/3T3 protein preparation. (D) Fluorograph of same gel as in (C). Arrows indicate location of p37/4.78 and p41/4.75. Drawn circles show location on fluorogram of added tropomyosin identified on stained gel (tm).

Cytoplasm from cells labeled with [^{3}H]leucine was heated at 100°C for 10 min, and precipitated proteins were removed by centrifugation. Although most proteins were eliminated by this treatment, p37/4.78 and p41/4.75 remained in the SOL fraction, demonstrating their heat stability (Fig. 9A and B).

When radiolabeled proteins from NIH/3T3 cells were mixed with purified rabbit skeletal muscle tropomyosin, the radiolabeled p37/4.78 and p41/4.75 peptides migrated in very close proximity to the Coomassie blue-stained tropomyosins, but were not identical to them (Fig. 9C and D). The slight differences in two-dimensional electrophoretic mobility are consistent with expected species differences in primary amino acid sequence of these proteins in mouse and rabbit as well as with the fact that skeletal muscle tropomyosins were compared with presumptive nonmuscle tropomyosins.

On the basis of these results we concluded that p37/4.78 and p41/4.75 are tropomyosins.

Effects of varying cell density. All of the preceding experiments were performed using cells growing logarithmically at 50 to 70% confluence. To determine whether synthesis of p37/4.78 and p41/4.75 tropomyosins was responsive to cell culture conditions such as crowding or contact inhibition, newly synthesized proteins of NIH/3T3 and DT cells were analyzed using cultures whose densities ranged from <25% confluence to full confluence. No differences in synthesis of p37/4.78 and p41/4.75 due to culture density were observed (data not shown). All comparisons between NIH/3T3 and DT with respect to synthesis of p37/4.78 and p41/4.75 were similar to that shown in Fig. 3.

Cells transformed by papovaviruses. To examine the possibility that the suppression of synthesis of tropomyosins might be characteristic of transformation by agents other than retroviruses, we studied the proteins synthesized by SV-3T3 and Py6-JM cells, two lines of 3T3 cells transformed by the papovaviruses SV-40 and polyomavirus, respectively. These cells exhibit a transformed phenotype with features similar to that of retroviral transformed cells, including loss of contact inhibition with piling up of cells, spindle-shaped morphology, colonial growth in semisolid medium, and tumorigenesis in nude mice. Their growth pattern in cell culture is virtually indistinguishable from that of retrovirally transformed DT cells.

Analysis of newly synthesized proteins in these cells by two-dimensional electrophoresis showed no evident suppression of synthesis of p37/4.78 and p41/4.75 in either case (Fig. 10). We were unable to confirm the observations of Matsumura et al. (29), who reported that other members of the tropomyosin group showed alterations in SV-40-transformed rat cells. Thus, although some modification of tropomyosin synthesis may be universal in imposing the transformed phenotype, the specific changes in synthesis of p37/4.78 and p41/4.75 described above appear to be restricted to oncogenesis by retroviruses.

Of the other proteins whose synthesis was generally suppressed in transformed lines and restored in revertants (Table 4), only p21/4.8 showed some inhibition in polyoma-

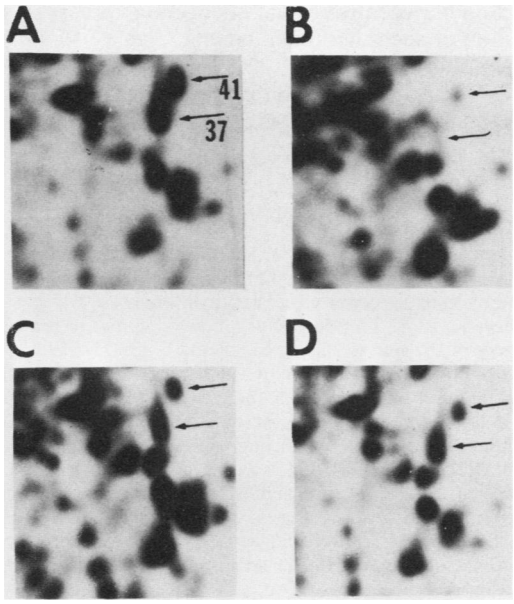

FIG. 10. Synthesis of p37/4.78 and p41/4.75 in papovavirus-transformed NIH/3T3 cells. Lines of NIH/3T3 cells transformed by papovaviruses SV-40 and polyomavirus as well as DT (v-*ras*^{Ki}-transformed) and NIH/3T3 cells were radiolabeled for 4 h with [^{3}H]leucine. Whole cell proteins were analyzed by two-dimensional electrophoresis and fluorography. (A) NIH/3T3 cells. (B) DT cells. (C) SV-3T3 cells. (D) Py6-JB cells.

transformed cells. None were suppressed in SV-40 transformants.

DISCUSSION

Previous studies using two-dimensional electrophoresis to examine proteins synthesized by virally transformed cells have detected numerous changes, generally reductions, in synthesis of specific proteins in particular lines of transformed cells (6, 18). Because of the large number of changes, and because of the possibility of random selection during prolonged culture of transformed cell lines, the relevance of any particular change to the transformation process is difficult to assess.

Generally, investigators have been concerned with identification of the immediate biochemical targets of particular oncogene products. Since a variety of unrelated oncogene products exists, an array of primary targets may be anticipated. Noda et al. (32) showed that retroviral oncogenes may be divided into two classes, those in which transforming activity is suppressed by fusion of transformed cells with the v-*ras*^{Ki}-carrying revertant lines C-11 and F-2 and those in which it is not. Of the transformed cell lines studied in the present work, those transformed by v-*ras*^{Ha}, v-*fes*, and v-*src* were suppressed by fusion with revertant line C-11. Cell lines transformed by v-*mos*, v-*fms*, SV-40, and polyomavirus were not suppresed by such fusion. This is consistent with the expectation of multiple primary targets, since it implies that the primary target of the oncogene product in the suppressible class lies proximal to the alteration that causes reversion, whereas in the nonsuppressible class it lies distal to it. However, despite the probable multiplicity of primary targets, the end result of the transformation sequence in cell culture is fairly uniform for many oncogenes as well as for DNA viruses and for chemical and spontaneous transformants, including loss of contact inhibition of growth and piling up of cells, change in cell morphology to a more rounded or spindle-shaped configuration, ability to grow as colonies in semisolid medium, and tumorigenicity in nude mice. This suggests that the various pathways affected by the initial action of oncogene products may converge on a limited number of common targets whose modification contributes to imposing the transformed phenotype.

To identify possible common target proteins whose synthesis may be altered by the action of many or all oncogenes, we have compared two-dimensional electropherograms of newly synthesized proteins from cell lines transformed by a variety of oncogenes, from cellular revertant lines, and from a line which expresses the v-*ras* oncogene in response to corticosteroids. In this way, we eliminated many changes which appear to be specific for particular cloned cell lines. Comparison of six cell lines transformed by v-*ras*^{Ki} reveals that most alterations in synthesis of specific proteins detected by this approach are not consistent concomitants of transformation by this oncogene and therefore are probably unrelated to the transformation process.

Our analysis revealed seven proteins whose synthesis was consistently suppressed in established cell lines transformed by each of the six different retroviral oncogenes we investigated and which were fully or partially restored in two revertant cell lines derived from one of the transformed lines studied. The finding of consistently suppressed synthesis in transformed cells and restored synthesis when these cells reverted to the nontransformed phenotype argues that synthesis of these seven proteins is required for expression of the nontransformed pattern of growth. Conversely, it appears that suppression of synthesis of these proteins is a biochemical consequence of expression of a number of structurally diverse oncogenes which is associated with establishment of the transformed state.

Two of these seven proteins were of special interest, since they were the only ones of this group whose suppression was correlated with the initial appearance of morphological alteration during corticosteroid-induced expression of v-*ras*^{Ha} in 433.3 cells under conditions in which the possibility of selective artifacts was minimized. These proteins (p37/4.78 and p41/4.75) were identified as tropomyosins, a family of at least five CSK proteins involved in microfilament structure (reviewed in references 28 and 30). Thus, our evidence for a common biochemical event which may be associated with transformation by a number of structurally diverse retroviral oncogenes is strongest for suppression of synthesis of p37/4.78 and p41/4.75. Our ability to identify these proteins as tropomyosins has considerable heuristic value.

In a study of the production of tropomyosins in transformed chick cells, Hendricks and Weintraub (20) reported reduced synthesis after transformation by Rous sarcoma virus. Similarly, in an investigation of the role of CSK elements in defining cell morphology, Leonardi et al. (26) reported that v-*ras*^{Ki} transformation of NRK (normal rat kidney) fibroblasts was associated with loss of detectable synthesis of two tropomyosins. Matsumura et al. (29, 30), also working with rat cells to study CSK structures and their control, reported similar results with Kirsten-transformed cells and noted that such changes may occur in mouse cells as well. They also reported that at least five proteins were involved in this group and that a different set was affected in SV-40-transformed rat cells.

These workers began their studies with a specific interest in CSK proteins and tropomyosins, and their observations were accordingly limited to those proteins. Our work proceeded from a general examination of proteins synthesized

by transformed cells and led us to the tropomyosins. Our work thus extends the findings of the earlier workers and reveals the particular importance of suppression of tropomyosin synthesis among a reasonably small number of consistent changes in synthesis of specific proteins in retroviral oncogenesis. Of all the alterations in protein synthesis which we were able to detect by two-dimensional gel analysis of cells transformed by a variety of related and unrelated retroviral oncogenes, only synthesis of the p37/4.78 and p41/4.75 tropomyosins was suppressed in all established transformed lines examined, was clearly correlated with the onset of morphological alteration, and was restored in cells reverting to the control growth pattern. Thus, our findings not only document the strong association of the level of synthesis of tropomyosins p37/4.78 and p41/4.75 with the prevailing pattern of cell growth but also serve to limit the number of other possible changes of similar importance. The results suggest that the biochemical pathways by which a number of structurally distinct retroviruses cause oncogenic transformation converge on a limited number of common targets, among which is the mechanism which regulates the synthesis of a subset of the family of tropomyosins.

Transformed cells show characteristic changes in cell morphology, becoming more rounded or spindle-shaped and less adherent to surfaces. These changes are associated with well-known alterations in the morphology of various CSK elements (28). It may be argued that these CSK changes, together with the suppression of synthesis of tropomyosin, a CSK-associated protein, are part of a nonspecific response on the part of the regulatory mechanisms of the cell to being forced to grow with the transformed phenotype. However, our observations with SV-40- and polyomavirus-transformed cells render that possibility unlikely. These cells, although manifesting morphological changes quite similar to those seen in cells transformed by retroviral oncogenes, show no suppression of p37/4.78 and p41/4.75 synthesis. Thus, such suppression is not a generalized cellular response to the transformation process but is a biochemical event which is specifically associated with expression of a number of retroviral oncogenes. The results also indicate that although suppression of synthesis of p37/4.78 and p41/4.75 tropomyosins may be a common pathway for retroviral oncogene effects, such suppression is not obligatory for transformation by all modalities. It remains to be determined whether other biochemical events, theoretically distal to tropomyosin suppression in the transformation sequence, will be elucidated which are common to the expression of both retroviral oncogenes and papovavirus-transforming genes.

The function of tropomyosin in nonmuscle cells is poorly understood. Molecules of tropomyosin bind to actin microfilaments in a regular fashion (11, 29, 37). It has been suggested that they may contribute to stability of the filament (34) possibly by antagonizing the action of components which promote dissassembly (3, 17, 29, 30). Unfortunately, the function of microfilaments themselves in nonmuscle cells is also only dimly perceived, being generally assigned a role in maintenance of cell configuration, motility, and adherence to surfaces (reviewed in references 8 and 28). CSK elements in general and microfilaments in particular are well known to be disrupted or deformed in transformed and neoplastic cells, and the possibility that such derangements may be causal in transformation has been frequently proposed (24, 28, 29, 33, 41). Our demonstration of the widespread suppression of tropomyosin synthesis by retroviral oncogenes together with previous work showing this effect in specific systems (20, 21, 26, 29, 30) may provide a biochemical connection between retroviral oncogene expression and the production of such disruptive effects.

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