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Post-Translational Regulation of the 54K Cellular Tumor Antigen in Normal and Transformed Cells

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The 54K cellular tumor antigen has been translated in vitro, using messenger ribonucleic acids from simian virus 40 (SV40)-transformed cells or 3T3 cells. The in vitro 54K product could be immunoprecipitated with SV40 tumor serum and had a peptide map that was similar, but not identical, to the in vivo product. The levels of this 54K protein in SV3T3 cells were significantly higher than those detected in 3T3 cells (D. I. H. Linzer, W. Maltzman, and A. J. Levine, Virology **98**:308-318, 1979). In spite of this, the levels of translatable 54K messenger ribonucleic acid from 3T3 and SV3T3 cells were roughly equivalent or often greater in 3T3 cells. Pulse-chase experiments with the 54K protein from 3T3 or SV3T3 cells demonstrated that this protein, once synthesized, was rapidly degraded in 3T3 cells but was extremely stable in SV3T3 cells. Similarily, in an SV40 *tsA*-transformed cell line, temperature sensitive for the SV40 T-antigen, the 54K protein was rapidly turned over at the nonpermissive temperature and stable at the permissive temperature, whereas the levels of translatable 54K messenger ribonucleic acid at each temperature were roughly equal. These results demonstrate a post-translational regulation of the 54K cellular tumor antigen and suggest that this control is mediated by the SV40 large T-antigen.

Animals bearing simian virus 40 (SV40)-induced tumors produce antibody to two virus-coded proteins, the large T-antigen (24) and the small t-antigen (3, 20), and a cellular protein of about 54,000 molecular weight (54K) (2, 9, 10, 12, 16, 23). The 54K protein has been detected in mouse 3T3 cells at about 2% the levels expressed in SV40-transformed 3T3 cells (13). SV40 infection of 3T3 cells results in a 20- to 30-fold increase in the levels of detectable 54K protein in these cells (12, 13). The SV40 *A* gene product, or large T-antigen, is required for this stimulation in the level of the cellular 54K protein in both virus-infected and -transformed mouse cells (13). Similar or identical proteins of 53,000 to 55,000 molecular weight have been detected in murine chemically transformed cell lines (4), embryonal carcinoma cell lines (12), spontaneously transformed cells (4; W. Maltzman, M. Oren, and A. J. Levine, Virology, in press), and lymphocytes from irradiation-induced leukemia (4). Both immunologically and chemically related 54K proteins have been detected in SV40-transformed rat, hamster, monkey, and human cell lines (6, 22). Thus, the expression of high levels of the 54K protein appears to be correlated with transformation (although not every transformed cell line expresses detectable levels of this protein), and the 54K protein appears to have been conserved, in part, over evolutionary time scales.

The mechanism by which the SV40 T-antigen increases the levels of the 54K cellular protein in virus-infected or -transformed cells is at present unclear. The SV40 *A* gene product might stimulate the de novo synthesis of the 54K protein, as it apparently does for several cellular enzymatic activities (8, 19) and the cellular ribosomal and transfer ribonucleic acid (RNA) genes (7, 25). Alternatively, the SV40 T-antigen might act at a post-translational step to stabilize a rapid turnover of the 54K protein in infected or transformed cells. This idea gains some creditability because of the observation that SV40 T-antigen is physically complexed, in solution, with the cellular 54K protein (10, 12, 15). Thus, the complex between SV40 T-antigen and the 54K protein could lead to a reduced turnover of the 54K protein in SV40-transformed cells when compared with 3T3 cells.

The experiments presented in this communication demonstrate that post-translational turnover of the 54K protein does indeed play a role in regulation of the differential levels of this protein in several SV40-transformed murine cell lines. Furthermore, the turnover rate appears to be dependent on the presence of functional SV40 T-antigen in the cells. Pulse-chase experiments indicate that the half-life of the 54K protein is much shorter in 3T3 cells than in SV3T3 cells. The observations presented here are consistent with the differential levels of the 54K protein

detected previously (13) and suggest a mechanism that can account for them.

MATERIALS AND METHODS

Cells. BALB/c 3T3 cells and the SV40-transformed BALB/c 3T3 derivative, SV40 T-2 (1), were provided by G. Todaro. SVTERtsA58Cb is an SV40-transformed murine teratocarcinoma-derived cell line (14). Primary mouse fibroblasts were prepared from the skins of prenatal BALB/c mice, passaged twice in NCTC 109 medium (Microbiological Associates) supplemented with 10% fetal calf serum and subsequently adapted to Dulbecco-modified Eagle medium containing 10% fetal calf serum.

Preparation of labeled cell extracts. The procedure for preparation of labeled cell extracts was as described previously (13). The labeling period was 4 h unless otherwise specified.

Cell fractionation and extraction of RNA. Cells were pelleted in phosphate-buffered saline and lysed by pipetting for 5 min at 0°C in lysis buffer [0.5% Nonidet P-40, 150 mM NaCl, 10 mM tris(hydroxymethyl)aminomethane-chloride (pH 7.4), 1 mM (ethylenediaminetetraacetate]. Nuclei were pelleted (Beckman TJ-6, 1,100 rpm, 4 min), and the pellet was reextracted with lysis buffer. The two supernatants were combined and incubated at 25°C for 1 to 2 h in a buffer containing 15 mM KCl–75 mM NaCl–0.25% Nonidet P-40–5 mM tris(hydroxymethyl)aminomethane-chloride (pH 7.4)–0.5 mM ethylenediaminetetraacetate–1.5% sodium dodecylsulfate (SDS)–100 to 200 μg of predigested proteinase K per ml. RNA purification was as described previously (5). All solutions and glassware used with RNA were pretreated with 0.3% diethylpyrocarbonate whenever possible.

RNA fractionation. Polyadenylic acid-containing RNA was selected by chromatography over oligodeoxythymidylic acid-cellulose. Further fractionation was carried out by sedimentation through a 15 to 30% sucrose gradient in 10 mM tris(hydroxymethyl)-aminomethane-acetate (pH 7.4)–0.1 M NaCl–0.1 mM ethylenediaminetetraacetate.

In vitro protein synthesis. Nuclease-treated rabbit reticulocyte lysate was prepared according to standard procedures (18). In vitro protein synthesis reaction mixtures contained 0.9 mM magnesium acetate, 160 mM potassium acetate, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.5), 0.2 mM guanosine triphosphate, 2 mM dithiothreitol, 74 μM spermine, 1 mM adenosine triphosphate, 8 mM creatine phosphate, 64 μg of creatine phosphokinase (type I, Sigma Chemical Co.) per ml, 50 μg of bovine transfer RNA per ml (gift of B. Dudock), 40 μM amino acids minus methionine, and 0.4 to 2.0 mCi of [^{35}S]methionine per ml (900 to 1,100 Ci/mmol; New England Nuclear Corp.). Incubation was at 30°C for 1 to 2 h.

Immunoprecipitation. Immunoprecipitation was as described previously (13). Before processing of in vitro protein synthesis samples, the reaction was stopped by addition of ethylenediaminetetraacetate to 10 mM and dithiothreitol to 1 mM, followed by centrifugation (17). Immunoprecipitated material was analyzed by electrophoresis on 15% SDS-polyacrylamide gels (13). Gels were processed by the procedure of Laskey and Mills (11) and quantitated as described in reference 13.

RESULTS

Fractionation of the 54K mRNA. The goal of these experiments was to develop an assay for the detection and purification of the messenger RNA (mRNA) that codes for the cellular 54K tumor antigen. To do this, RNA was extracted (see above) from cells of the SV40-transformed line SVT2, and the polyadenylic acid-containing RNAs were selected by chromatography over an oligodeoxythymidylic acid-cellulose column. The RNA was then sedimented through a sucrose gradient and fractions were collected. Each fraction was then added to a reticulocyte in vitro translation system to prime protein synthesis. The [^{35}S]methionine-labeled products were immunoprecipitated by using sera either from mice carrying tumors induced with SV40-transformed cells (tumor sera) or from normal mice (normal sera). These immunoprecipitates were collected and analyzed by SDS-polyacrylamide gel electrophoresis. Figure 1 presents the autoradiogram of such a gel. The SV40 large T-antigen (94,000 molecular weight) was synthesized in vitro in the mRNA fractions that sedimented at about 19S, and this antigen was immunoprecipitated with tumor sera but not with normal sera. A 54K protein was synthesized in vitro and immunoprecipitated with tumor sera (and not normal sera) from an mRNA fraction that sedimented at about 17S.

Peptide maps of 54K synthesized in vivo and in vitro. The 54K protein synthesized in vitro was selectively immunoprecipitated with tumor sera, but not with normal sera, and was of the same molecular weight as the in vivo protein. To determine whether this 54K protein synthesized in vitro was the same protein detected with tumor sera in vivo, peptide mapping of the [^{35}S]methionine-containing tryptic peptides was carried out. The in vivo synthesized 54K protein was labeled with [^{3}H]methionine in cell cultures of SVT2 cells and purified by immunoprecipitation and gel electrophoresis (Maltzman et al., in press). The mixture of the in vitro [^{35}S]methionine-labeled protein (mRNA from SVT2 cells) and the [^{3}H]methionine in vivo labeled 54K protein was cleaved with trypsin, and the peptides were chromatographed on a Chromobead (Technicon) cation-exchange column. Figure 2 presents the chromatographic profile obtained with the tryptic peptides from these two proteins. Seven or eight of the peptides from the in vivo and in vitro products were

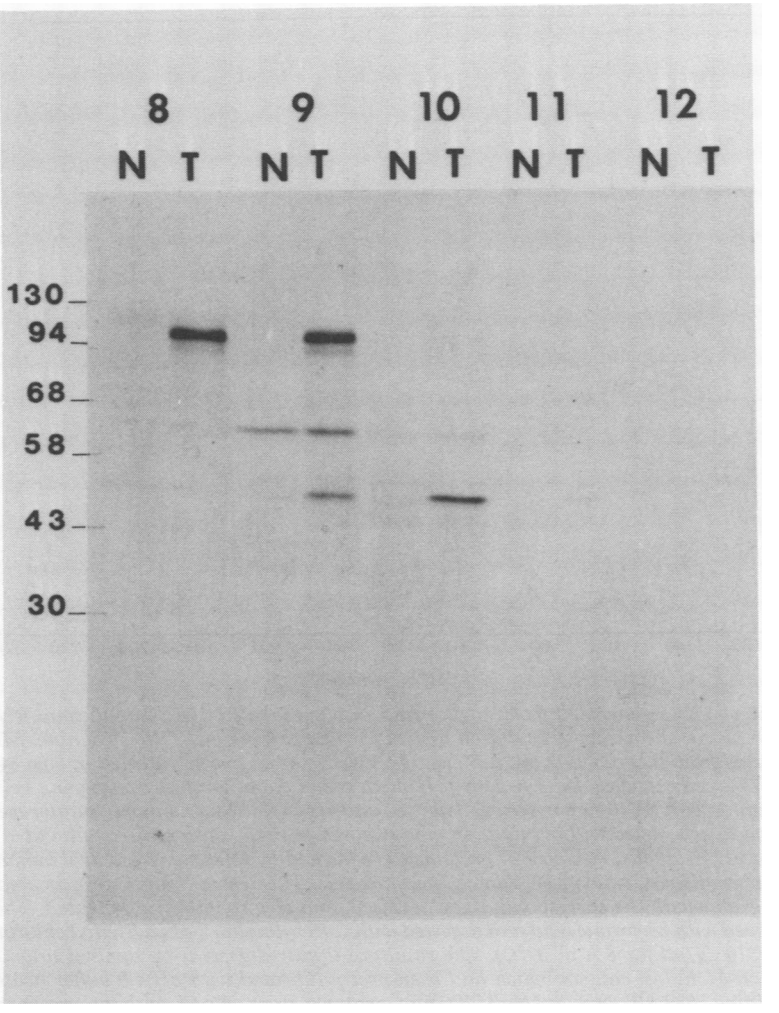

FIG. 1. *Translation products of sucrose gradient-fractionated SV3T3 mRNA. Cytoplasmic RNA was extracted from SVT2 cells and selected over oligodeoxythymidylic acid-cellulose as described in the text. The polyadenylic acid-containing RNA was fractionated over a 15 to 30% sucrose gradient, and 19 fractions were collected. The peaks of 28S and 18S ribosomal RNA appeared in fractions 5 and 9, respectively. RNA from the indicated fractions was ethanol precipitated and used to prime in vitro protein synthesis. The reaction products were immunoprecipitated with serum from either normal (N) or tumor-bearing (T) mice and analyzed by electrophoresis on an SDS-polyacrylamide gel. Numbers on the left denote the apparent molecular weights ($\times 10^3$) of the polypeptides migrating to the indicated positions.*

identical. Two to four additional peptides were present in the in vivo product ([^{3}H]methionine label). Since the in vivo and in vitro products have similar apparent molecular weights in SDS-polyacrylamide gels, these extra peptides may result from some fraction of the 54K proteins in vivo undergoing post-translational modifications (Maltzman et al., in press). The in vitro and in vivo 54K proteins are clearly closely related polypeptides and may well be identical in primary amino acid sequences based upon both the immunological and the peptide map analyses.

Levels of 54K mRNA in 3T3 and SV3T3 cells. When 3T3 cells and SV40-transformed 3T3 cells were labeled for 4 h with [^{35}S]methionine and the 54K proteins were isolated by immunoprecipitation, it was found that the SV3T3 cells contained substantially more 54K protein than the 3T3 cells (13). To determine whether this was also the case with the levels of 54K mRNA, the following experiment was per-

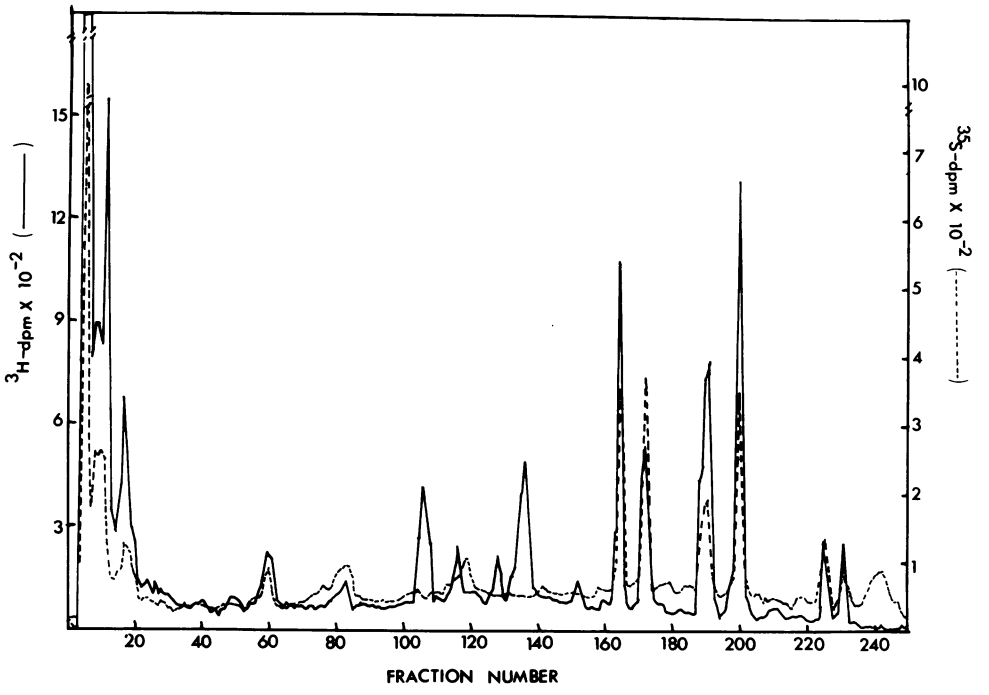

FIG. 2. *Tryptic peptides generated from in vivo and in vitro labeled 54K. Cytoplasmic RNA from SVT2 cells was used to prime an in vitro translation system in the presence of [^{35}S]methionine. 54K protein was immunoprecipitated from this reaction mixture, using SV40 tumor serum. ^{3}H-labeled in vivo 54K protein was isolated from SVT2 cells labeled for 4 h with [^{3}H]methionine (25 μCi/ml), by preparing cell extracts and immunoprecipitating with SV40 tumor serum. The ^{35}S- and ^{3}H-labeled immunoprecipitates were run on 15% SDS-polyacrylamide gels, dried without fixation, and exposed to high-contrast X-ray film at −70°C. The ^{35}S-labeled in vitro and ^{3}H-labeled in vivo 54K proteins were located by reference to control immunoprecipitates with tumor and normal sera, which were run on the same gels. The desired bands were located by alignment of the autoradiograms with the corresponding gel, excised, and electrophoretically eluted. The 54K proteins eluted were oxidized with performic acid and digested with L-(tosylamido 2-phenyl)ethyl chloromethyl ketone-treated trypsin (310 μg/ml for 8 h at 37°C). The resulting tryptic digests were applied to a Chromobead P (Technicon) cation-exchange column which had been preequilibrated with pH 1.9 buffer (water-acetic acid-formic acid-pyridine, 589:316:88:8, vol/vol). Tryptic peptides were eluted with an exponential gradient composed of pH 1.9-pH 1.9-pH 4.5 buffer (water-pyridine-acetic acid-formic acid, 653:158:140:39, vol/vol) at 65°C. Fractions, 2.5 ml, were collected, dried at 85°C, dissolved in 0.1 ml of glacial acetic acid, and counted in 4.5 ml of Triton-based scintillation cocktail. For scintillation counting a Beckman LS7500 scintillation counter was used, with a data reduction program which subtracted background counts, calculated crossovers, and converted counts per minute to disintegrations per minute.*

formed. Two cell cultures of 3T3 and SV3T3 cells were labeled with [^{35}S]methionine for 4 h, and the soluble proteins were extracted and immunoprecipitated with SV40 tumor sera. A second set of 3T3 and SV3T3 cell cultures was used to extract the cytoplasmic RNA, which was then used to prime the in vitro synthesis of SV40 T-antigen and the 54K protein. The levels of RNA from 3T3 and SV3T3 added to the in vitro synthesizing system were optimized so that each sample gave the maximum total incorporation of [^{35}S]methionine counts per minute into trichloroacetic acid-precipitable proteins. Equal amounts of trichloroacetic acid-precipitable radioactivity incorporated in vitro or in vivo were immunoprecipitated and analyzed by SDS-polyacrylamide gel electrophoresis. In this experiment 32 μg of 3T3 RNA and 35 μg of SV3T3 RNA were used to prime the reaction samples taken for analysis. The autoradiogram of such a gel is presented in Fig. 3. SV3T3 cells labeled in vivo contained the SV40 T-antigen and the 54K protein, whereas the 3T3 cells labeled in vivo had much smaller levels (5- and 12-fold less in duplicate experiments) of the 54K protein, in agreement with previous results (13). However, the levels of the in vitro translated 54K protein primed with RNA from 3T3 cells were 1.5-fold greater than those of the 54K protein synthesized in vitro with comparable levels of SV3T3

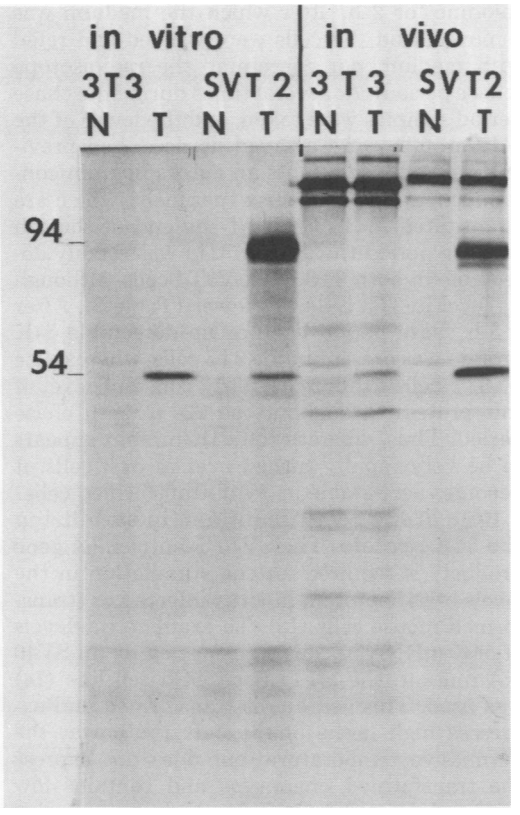

FIG. 3. *Comparison of 54K mRNA and protein levels in 3T3 and SV3T3 cells. Parallel cultures of 3T3 and SV3T3 cells were either labeled with [^{35}S]methionine or used to extract total cytoplasmic RNA. The RNAs were translated in vitro, and equal amounts (13 × 10^6 cpm) of trichloroacetic acid-insoluble radioactivity from either the in vitro reactions or the in vivo labeled cell extracts were immunoprecipitated and analyzed on an SDS-polyacrylamide gel. The samples taken for analysis represent the in vitro products of 32 and 35 μg of 3T3 and SV3T3 RNA, respectively. The immunoprecipitation reactions with in vitro synthesized proteins also included an amount of unlabeled 3T3 cell extract comparable to that present in the reactions utilizing labeled cellular extracts. For definitions of N and T, see legend to Fig. 1.*

mRNA (Fig. 3; Table 1, experiments 1 and 2). Thus, the difference in the levels of 54K protein detected in 3T3 cells by labeling proteins in vivo is not congruent with a similar difference in the levels of translatable 54K mRNA extracted from these cells.

To ascertain that the amount of in vitro synthesized 54K protein detected by immunoprecipitation can indeed be used as a measure of the amount of specific mRNA present, a dose-response experiment was carried out. Increasing amounts of cytoplasmic RNA from 3T3 and SV3T3 cells were translated and analyzed as described previously. The relative level of 54K protein detected in each in vitro translation was calculated from densitometer tracings of the autoradiograms (see above) and plotted as a function of the RNA level used to prime the reaction. Figure 4 presents these data and clearly indicates that mRNA is indeed limiting in this reaction and that the levels of the 54K protein detected by this procedure reflect the levels of corresponding mRNA in the preparation. A similar result was obtained with the translation of the SV40 T-antigen messenger in vitro, using RNA extracted from the SVT2 cells (data not presented).

TABLE 1. *Relative levels of the 54K protein in 3T3 and SV3T3 cells synthesized in vivo and in vitro*[a]

Expt	Source of Protein	Relative Level (%) of 54K
1	3T3, in vivo	8.6
	SV3T3, in vivo	100
	3T3, in vitro	159.3
	SV3T3, in vitro	100
2	3T3, in vivo	22.1
	SV3T3, in vivo	100
	3T3, in vitro	156.3
	SV3T3, in vitro	100
3	Mouse fibroblasts, in vivo	5.7
	SV3T3, in vivo	100
	Mouse fibroblasts, in vitro	48.3
	SV3T3, in vitro	100

[a] The 54K protein was immunoprecipitated from either [^{35}S]methionine-labeled cell extracts (in vivo) or reticulocyte lysates (in vitro). For experimental details, see the text and the legend to Fig. 3. Levels shown are expressed as percentage of the amount of 54K protein demonstrable with SV3T3 cells under a comparable set of conditions (in vivo or in vitro).

Although the 3T3 cells used in this study were carefully maintained and exhibited normal cell growth properties, one can still argue that 3T3 cells show an aberrant pattern of 54K protein regulation because they are a permanent cell line in culture. For this reason the experiment described previously was repeated, and the levels of 54K protein in vivo and translatable mRNA in vitro were quantitated, using SV3T3 cells and primary mouse fibroblasts derived from BALB/c mice. The results are presented in Table 1 and demonstrate that the levels of 54K protein labeled in vivo were 17.5-fold higher in SV3T3 cells than in the mouse fibroblasts, whereas the level of translatable mRNA was only about 2-fold higher in the SV3T3 cells.

Turnover of 54K protein in vivo. That

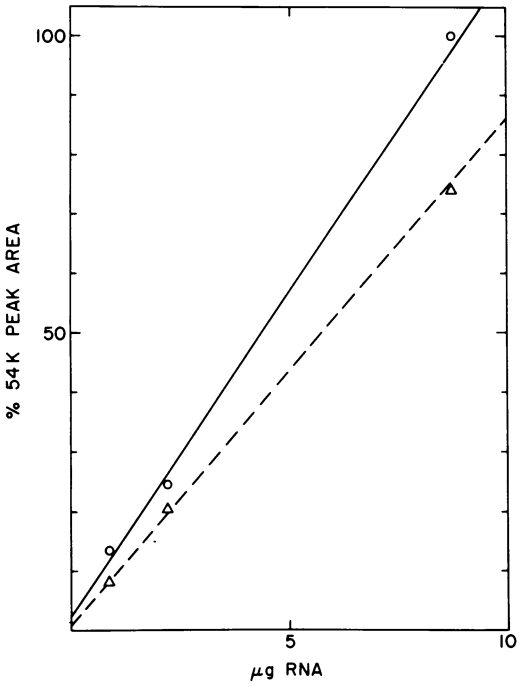

FIG. 4. *Correlation between the amount of RNA translated and the amount of 54K protein detected. Increasing amounts of total cytoplasmic RNA from either 3T3 (▲) or SV3T3 (○) cells were translated, and the reaction products were immunoprecipitated and analyzed as detailed before. The reaction saturated for RNA at about 17.5 μg of RNA (twice the amount used for the last point). The amount of 54K protein is expressed as percentage of the maximum value obtained in this experiment.*

comparable levels of RNA from 3T3 and SV3T3 cells translate about the same levels of 54K protein in vitro, but 5- to 12-fold differences in the level of 54K protein were observed by in vivo labeling of proteins and immunoprecipitation (Table 1), could be explained in several ways. For example, the mRNA for the 54K protein may be efficiently translated in vitro but specific inhibitors of translation in 3T3 cells or activators of translational efficiency in SV3T3 may be present in vivo. Alternatively, the 54K protein made in vitro and in SV3T3 cells may be efficiently immunoprecipitated, but in 3T3 cells most of this protein is in a form which is not recognized by the antibody. Yet another possibility is that SV3T3 cells and 3T3 cells may indeed have comparable levels of 54K mRNA, but the protein in 3T3 cells could be rapidly degraded (turnover), whereas in SV3T3 cells it may be stabilized and accumulated.

To test this latter idea a pulse-chase experiment was performed. Subconfluent cell cultures of 3T3 and SV3T3 were labeled with [^{35}S]methionine for 2 h, after which the medium was removed, and the cells were washed and refed with medium not containing the radioisotope (chase period). At several times during the chase period samples were taken, and the levels of the 54K protein were assayed as described previously. Figure 5 presents an autoradiogram containing the results of this experiment, which are quantitated in Table 2. At the end of the 2-h labeling period the 54K protein was readily detectable in both 3T3 and SV3T3 cells, although its level in 3T3 cells was lower (Table 2). After a 3-h chase period little or no detectable 54K protein was present in the 3T3 cells, whereas the SV3T3 cells exhibited a fairly constant level of this protein throughout the 22- to 24-h chase period. Thus, whereas the 54K protein appears to be very rapidly turned over in 3T3 cells, it becomes very stable in SV40-transformed cells.

Role of the SV40 T-antigen in stabilizing the 54K protein. The SV40 T-antigen (*A* gene product) is required for the stimulation in the levels of 54K protein in virus-infected or -transformed mouse cells (13). To examine the levels of 54K mRNA in cells transformed by an SV40 *tsA* mutant, the SVTERtsA58Cb cell line (14) was used. This cell line is transformed and expresses high levels of the 54K protein at the permissive temperature, but does not express the transformed phenotype and contains low levels of the 54K protein at the nonpermissive temperature (13, 14). SVTERtsA58Cb cells were grown at either 32 or 39.5°C and labeled with [^{35}S]methionine for 4 h, followed by a 20-h chase period at the same temperatures. One set of cell cultures kept at either temperature was utilized to extract cytoplasmic RNA for an in vitro translation reaction. A parallel set of cell cultures was processed for immunoprecipitation of the in vivo labeled proteins, which were then analyzed by SDS-polyacrylamide gel electrophoresis. The autoradiogram of such a gel is presented in Fig. 6 and these results are quantitated in Table 3. At the nonpermissive temperature the 54K protein labeled and chased in vivo was barely detectable (a 100-fold difference between 32 and 39.5°C). On the other hand, the levels of 54K mRNA, as assayed by in vitro translation of this protein, were not affected by the temperature differential or were even slightly increased at 39.5°C. It is noteworthy that the RNA from cells grown at 39.5°C programmed higher levels of SV40 T-antigen and t-antigen in vitro than equivalent levels of RNA from these cells kept at 32°C. This is in good agreement with the fact that the *tsA* mutation produces a faulty T-antigen which does not modulate down SV40 early transcription (21). Thus, the in vitro translation assay used here can detect different levels of

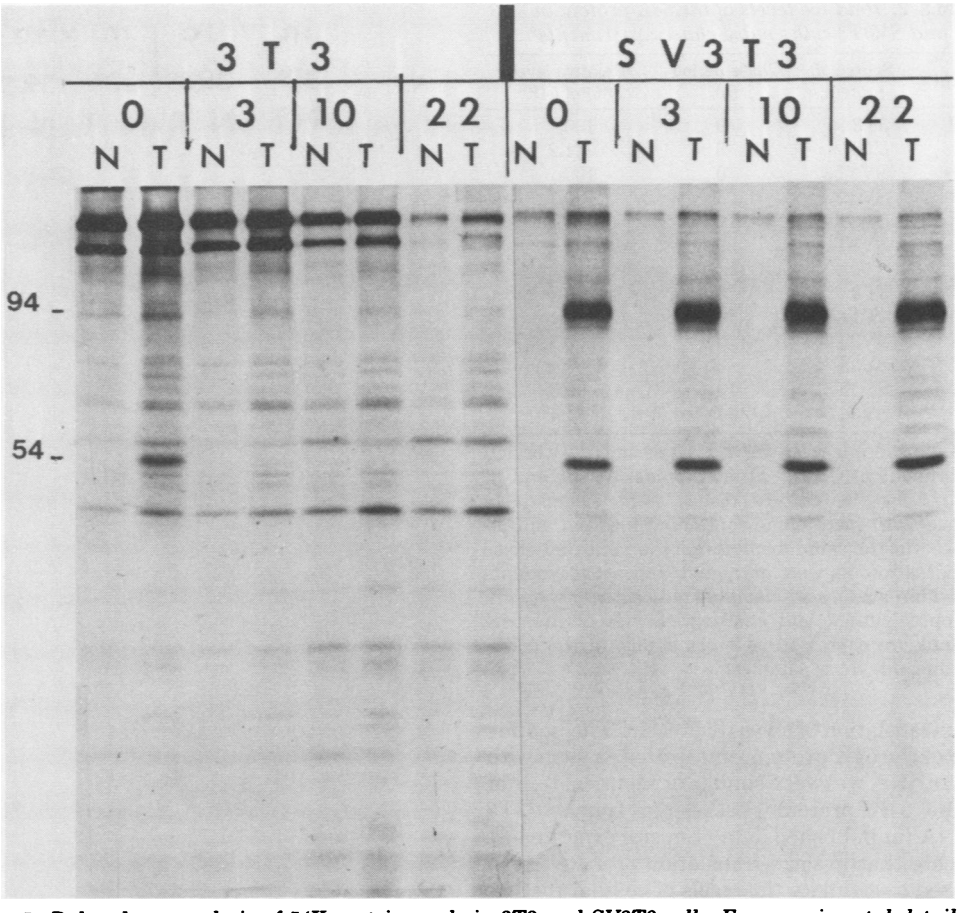

FIG. 5. *Pulse-chase analysis of 54K protein made in 3T3 and SV3T3 cells. For experimental details see footnote to Table 2 (experiment 1). Numbers refer to the length of the chase period (hours), N and T denote serum from either normal or tumor-bearing hamsters. The autoradiogram of the 3T3 cells was exposed for 5 days and that of the SV3T3 cells was exposed for 2 days.*

specific mRNA (T-antigen or 54K protein) in these cells.

This experiment demonstrates that a functional T-antigen is required to provide high levels of the 54K cellular protein in vivo. This regulation does not appear to operate at the level of transcription (translatable mRNA) but seems most likely to occur at the post-translational stage of protein turnover.

DISCUSSION

mRNA extracted from 3T3 cells can be used to prime the in vitro synthesis of SV40 T-antigen and a 54K cellular tumor antigen. Evidence that the 54K proteins synthesized in vivo and in vitro are closely related is as follows: (i) SV40 tumor serum but not normal serum immunoprecipitates the in vivo and in vitro proteins; (ii) the apparent molecular weights on SDS-polyacrylamide gel electrophoresis of the in vivo and in vitro proteins are similar or identical; and (iii) seven or eight of the [^{35}S]methionine-containing tryptic peptides generated from the in vivo and in vitro proteins are identical. The 54K protein synthesized in SV3T3 cells in vivo had two to four extra [^{35}S]methionine-containing tryptic peptides, when compared to the in vitro translated 54K protein synthesized from mRNA derived from these same cells. Because the in vivo and the in vitro synthesized proteins have very similar molecular weights, it appears likely that these extra peptides, derived from the protein made in vivo, could be due to post-translational modifications. The peptide maps of the 54K proteins from embryonal carcinoma cells or 3T12 cells are identical to the in vitro translated 54K protein made from SV3T3 mRNA (Maltzman et al., in press).

Surprisingly, RNA from SV3T3 and 3T3 cells had roughly equal efficiencies of priming the in

TABLE 2. *Relative levels of the 54K protein in 3T3 and SV3T3 cells: pulse-chase experiments*[a]

Expt	Source of protein	Chase period (h)	Relative level (%) of 54K
1	3T3	0	100
	3T3	3	<1.2
	SV3T3	0	100
	SV3T3	3	120.7
	SV3T3	10	125.4
	SV3T3	22	132.8
2	3T3	0	100
	3T3	3	4.1
	SV3T3	0	100
	SV3T3	3	98.4
	SV3T3	10	95.6
	SV3T3	24	84.4

[a] Cells were labeled for 2 h (experiment 1) or 1 h (experiment 2) with [^{35}S]-methionine. At the end of the pulses, the culture dishes were exhaustively washed with methionine-containing medium and incubated in the same medium for the indicated chase periods. Proteins were extracted, and equal amounts of trichloroacetic acid-insoluble radioactivity were immunoprecipitated and analyzed. Levels of the 54K proteins are expressed as a percentage of the corresponding 0-h chase samples.

vitro translation of the 54K protein. The peptide map of the 54K protein synthesized in vitro with 3T3 mRNA was very similar or identical to that of the 54K protein synthesized from SV3T3 mRNA (unpublished data). Several experiments indicate that in vitro translation of mRNA can be used to estimate the levels of cellular mRNA of a particular protein. First, the level of 54K protein and T-antigen synthesized in vitro was proportional to the level of input RNA in the reaction. Second, under conditions where higher levels of SV40 T-antigen mRNA were expected in vivo (SV40 *tsA* cells at 39.5°C), higher levels of T-antigen were detected in vitro. If indeed the levels of 54K mRNA are the same in 3T3 and SV3T3 cells, then additional information would be required to explain the differences in the levels of the 54K protein found in 3T3 and SV3T3 cells by labeling proteins in vivo. The pulse-chase experiments carried out with 3T3 and SV3T3 indicate that the half-life of the 54K protein in 3T3 cells is less than 1.5 h, whereas this protein is stable over a 24-h period in SV3T3 cells. Taken together, the results on the levels of 54K mRNA and these pulse-chase experiments strongly indicate that post-translational turnover of the 54K protein can, at least in part, account for the different levels of this protein detected in 3T3 and SV3T3 cells (13).

There are two reasonable mechanisms for the stabilization of the 54K protein in SV3T3 cells.

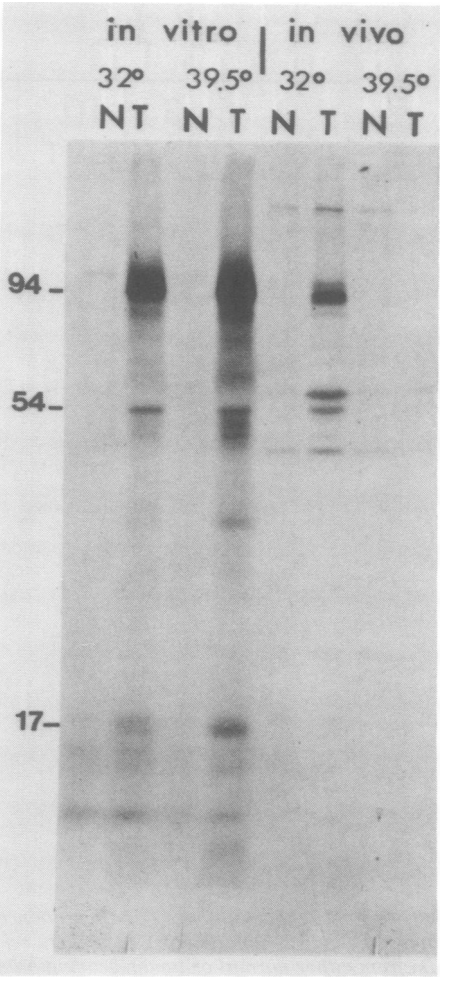

FIG. 6. *Comparison of 54K mRNA and protein levels in SVtsA-transformed cells grown at 32 or 39.5°C. SVTERtsA58Cb cells were grown at 32°C for 16 h, at which time half of the cultures were shifted to 39.5°C. After 3 days at 32 or 39.5°C, one dish of each group was labeled for 4 h with [^{35}S]methionine and chased for 20 h with nonradioactive medium, and proteins were extracted. The rest of the dishes were mock-labeled, chased, and used to prepare cytoplasmic RNA, which was then analyzed by in vitro translation. The experimental design was as outlined in the legend to Fig. 3, except that 12.4 × 10^6 cpm were included in each reaction. The in vitro products analyzed were synthesized under optimal incorporation conditions, using 23 and 25 μg of RNA extracted from cells grown at 32 and 39.5°C, respectively. For definitions of N and T, see legend to Fig. 1.*

First, by virtue of the physical complex between SV40 T-antigen and the 54K protein, the turnover of the latter might be reduced. Second, and not mutually exclusive of the first point, post-

TABLE 3. *Relative levels of the 54K protein in SVTERtsA cells at 32 and 39.5°C*[a]

Source of protein	Relative level (%) of 54K
SVTERtsA58Cb, 32°C, in vivo	100
SVTERtsA58Cb, 39.5°C, in vivo	0.8
SVTERtsA58Cb, 32°C, in vitro	100
SVTERtsA58Cb, 39.5°C, in vitro	150.8

[a] The 54K protein was immunoprecipitated from either [^{35}S]-methionine-labeled cell extracts (in vivo) or reticulocyte lysates in vitro. For experimental details, see the text and the legend to Fig. 6. The levels of 54K protein are expressed as a percentage of the amount of 54K detectable in cells at 32°C (in vivo or in vitro).

translational modification of the 54K protein could result in a reduced level of protein turnover. In either case, T-antigen is clearly required for this increased stability of the 54K protein (13). These results do not eliminate the possibility that transcriptional regulation may also play a role in some different situations or cell types in also regulating the levels of this 54K tumor antigen. In addition, some factors other than the SV40 T-antigen must regulate the higher levels of the 54K protein in embryonal carcinoma cells or 3T12 cells. It is tempting to postulate that the 54K protein has a regulatory function in normal cells which is compatible with its rapid turnover. By markedly decreasing the rate of turnover of the cellular 54K protein, the SV40 T-antigen could well begin a series of events that results in the transformed phenotype. The concept that viral proteins can regulate cellular functions by altering post-translational turnover rates of these proteins now appears to be established, and its role in cellular transformation will need to be investigated.

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