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Cells Transformed by a Wide Variety of Agents Express Higher Abundance Levels of Some Cellular RNA Species

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A cDNA-cloned library was prepared from mRNA synthesized by SV40-transformed mouse cells. Eleven cDNA clones were selected based on their ability to hybridize higher levels of mRNA in SV40-transformed 3T3 cells than in 3T3 cells. These cDNA clones were employed to screen the steady-state levels of cytoplasmic RNAs in a wide variety of viral (SV40, polyoma, adenovirus, and Rous sarcoma virus) and nonviral (methylcholanthrene, embryonal carcinoma) transformed cell lines. Two of the cDNA clones—A17 and 104—detected greater than 40–100-fold higher levels of mRNA in all the transformed cell lines tested when compared to nontransformed cells (3T3, C3HEF). The levels of mRNA complementary to these two cDNAs were regulated in a temperature-sensitive fashion (87–100-fold) in both SV40tsA- and RSV ts-src-transformed murine cell lines. These two cDNA clones detected greater than 100-fold, higher levels of complementary RNA derived from SV40 tumor tissue than in normal mouse liver. RNA species complementary to cDNA clones A17 or 104 were not detected in either actively growing nontransformed cells or in serum-stimulated 3T3 cells. The abundance levels of mRNAs detected by these two cDNA clones appear to be regulated 100-fold or greater by the transformed state, independent of the transforming agent. The higher levels of these RNA species detected in transformed mouse cells appear not to be solely regulated by the state of growth of nontransformed cells.

Key words: cytoplasmic RNA, messenger RNA, 3T3 cells, C3HEF, SV40

In a previous study [1] an SV40-transformed mouse cell line cDNA library was employed to obtain eleven cDNA clones that detected, by hybridization, much higher abundance levels of cellular RNAs from SV40-transformed cells than from their nontransformed counterparts. Three of these cDNA clones detected cellular RNA species in greater than 100-fold abundance in SV3T3 cells compared with 3T3 cells, in two independently derived SV40tsA-transformed cell lines at 32°C compared to nontransformed growing cells at 39.5°C, and in SV40 tumor tissue compared to

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normal tissue. The levels of cellular RNAs complementary to these cDNA clones increased dramatically after SV40 infection of nontransformed 3T3 cells in culture. Some of these cDNA clones (A17, 104, 397) detected greater than 100-fold higher levels of cellular RNA transcripts in all of the SV40-transformed cell lines tested when compared with nontransformed 3T3 cells or primary mouse fibroblasts. Other cDNA clones (403, 218, B50) detected cellular RNA species that were in higher abundance in some, but not all, SV40-transformed cell lines examined and compared with nontransformed cells. Finally, some cDNA clones detected equal and high levels of cellular RNA in both nontransformed and SV40-transformed cell lines studied [1].

This panel of eleven distinct cDNA clones complementary to cellular mRNAs provided the opportunity to investigate two related questions: 1) Are any of these mRNAs expressed in high abundance in a wide variety of transformed cell lines independent of the transforming agents? and 2) Are any of these mRNAs regulated by cellular growth independent of the transformed phenotype? The experiments described in this study detected three cDNA clones—A17, 104, and 218—whose transcripts were in 40 to 100-fold greater abundance in SV40, polyoma, adenovirus, RSV, methylcholanthrene, and embryonal carcinoma transformed cell lines compared to their nontransformed counterparts. The RNA levels complementary to cDNA clones A17 and 104 were regulated in a temperature-sensitive fashion (87–100-fold) in both SV40tsA- and RSV ts-src-transformed cell lines. No RNA complementary to cDNA clones A17 or 104 was detected in actively growing 3T3 cell cultures or serum-stimulated 3T3 cell cultures. Thus cDNA clones A17 and 104 appear to detect an enhanced abundance of transformation related RNAs in a wide variety of transformed cell lines.

MATERIALS AND METHODS

Cell Lines

BALB 3T3 cells and the SV40-transformed cell derivative SV40 BALB/c 3T3-T2 [2] were obtained from G. Todaro. The SVtsA58cb cell line is described by Maltzman et al [3]. The Py3T3 cell line is a polyoma-transformed BALB/c 3T3 cell line of the clone A31 derivative [2]. The AdDC-3 cell line is an adenovirus type 5-transformed C3H embryo fibroblast cell line as described by Sarnow et al [4]. The Rous sarcoma virus-transformed BALB/c 3T3 cell line [5] and F9 is an embryonal carcinoma cell line [6]. All cell lines were grown in 150-mm Falcon plastic dishes in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 20 mM glutamine, penicillin (100 μ/ml, streptomycin (100 μg/ml), and 10% heat-inactivated calf or fetal calf serum. SV40-induced tumors were obtained several weeks after a subcutaneous injection of SV40 SV BALB/c 3T3-T2 cells into BALB/c mice.

Nucleic Acid Reagents

RNA was extracted from cell cultures and tumor tissue as described elsewhere [1]. The cDNAs were grown in E coli JA221 in pBR322 vectors and the DNA was isolated as described [1]. RNA was labeled with polynucleotide kinase by a procedure described by Spradling et al [7] and modified by Schutzbank et al [1]. Dot-blot hybridization procedures were those of Thomas [8] and modified as described elsewhere [1]. The filters were counted in a liquid scintillation counter as described [1].

Serum Stimulation of 3T3 Cell Cultures

Cell cultures of 3T3 cells were allowed to grow to a confluent monolayer and then starved in 1% depleted serum containing medium for 24 hr. Fresh fetal calf serum (10%) was added at time 0. DNA synthesis began in the cultures at 10 hr and reached a maximum at 18 hr. Samples were taken for RNA extraction at 0, 2, 8, 12, and 18 hr.

RESULTS

Measurement of the Steady State Levels of RNA Species in Transformed and Nontransformed Cell Lines

Eleven cDNA clones—derived from mRNAs of an SV40-transformed cell line [1]—were employed to measure the levels of these mRNAs in a wide variety of transformed and nontransformed cell lines. Cytoplasmic RNA was extracted from the cell lines listed in Table I. The RNA was labeled with γ-ATP^{32} and polynucleotide kinase after the RNA was sheared by mild alkaline hydrolysis [7]. The labeled RNA was then hybridized to nitrocellulose filters containing one of the eleven cloned cDNAs that are complementary to different cellular mRNAs [1]. The nontransformed cell lines employed in the study were BALB/c 3T3, C3H embryo fibroblasts, an SV40tsA-transformed cell line at 39.5°C [2,3,4], and a RSV ts-src(LA90)-transformed BALB 3T3 line at 39.5°C. The transformed cell lines employed were BALB/c SV3T3-T2, SV40tsA at 32°C, BALB/c Py3T3, Ad DC3-C3H cells, RSV BALB/c 3T3 cells, RSV ts-src at 32°C, Meth A-BALB/c 3T3, and F9 embryonal carcinoma cells. All nontransformed and transformed cell cultures were actively growing when the cells were harvested and cytoplasmic RNA was extracted. Table I presents the ratios of the cpm hybridized with RNA from the transformed cell against that of RNA

TABLE I. Steady State Levels of RNA Species in Transformed and Nontransformed Cell Lines

	Ratio of cpm hybridized								
Clone no.	SV3T3 3T3	SVts[a] 32°/39°C	Py3T3 3T3	AdDC-3 C3HEF	RSV-3T3 3T3	RSV-ts LA90 32°/39°C	Meth A 3T3	F9 3T3	SV40 Tumor Liver
A17	> 100[b]	> 100	> 79	> 100	> 100	> 100	> 75	> 100	> 100
104	> 100	> 100	> 43	> 41	> 96	> 87	> 100	> 100	> 100
397	> 100	> 100	—[c]	6	—	—	—	—	> 100
403	> 100	> 100	—	> 100	—	—	—	—	> 100
218	> 100	> 100	> 100	> 100	> 100	4	> 100	> 100	> 100
B50	> 100	> 100	2	3	11	7	11	4	> 100
105	> 100	10	3	> 73	14	> 97	15	20	> 100
192	> 20	8	5	6	1	6	5	21	5
348	16	4	19	7	36	3	36	60	30
85	5	6	2	4	2	9	1	14	7
285	2	1	8	2	2	4	7	4	3

[a]The SV40tsA-transformed cell line employed was SVtsA58cb.
[b]The sign > indicates that the divisor in each case contained no detectable cpm above background.
[c]The dash indicates that the numerator in each case contained no detectable cpm above background.

from its nontransformed counterpart for each of the eleven cDNA clones. Clones A17 and 104 detected between 40–100-fold higher levels of cellular RNA in transformed cells than in nontransformed cells. In cell lines SV40tsA and RSV ts-src the levels of these cellular transcripts were regulated with a temperature shift, ie, higher abundance at 32°C (transformed) than at 39.5°C (nontransformed). 3T3 cells grown at 32°C or 39.5°C contained no detectable RNA species complementary to clone A17 or 104, eliminating temperature as the sole determining variable. RNA from an SV40-transformed cell line-induced tumor in a mouse also contained greater than 100-fold more RNA complementary to clones A17 and 104 than did mouse liver tissue. The cellular transcripts complementary to clone 104 and A17 appear to be elevated 40–100-fold in transformed cells, compared to nontransformed cells, independent of the transforming agent employed. RNAs complementary to clones 397, 403, and B50 were consistently present in 100-fold or more higher concentrations in SV40-transformed cells and tumor tissue when compared to their nontransformed cell counterparts. The higher levels of these RNAs were regulated in a temperature-sensitive fashion in SV40tsA-transformed cells. However, the RNA species complementary to these cDNA clones were either not detectable in other transformed cells (cDNA 397, 403 in Py, RSV, Meth A, F9), or if present, were not in higher concentrations in the transformed cell compared to the nontransformed cell lines (cDNA B50, in Py, Ad, RSV, Meth A, F9). Thus, the RNA species complementary to cDNA's 397, 403, and B50 appear to be regulated or present in enhanced levels only in the SV40-transformed cell lines.

RNA species complementary to cDNA clone 218 were found in greater than 100-fold more abundant levels in all of the transformed cell lines compared with their nontransformed counterparts. The only exception to this was that the RNA complementary to clone 218 c-DNA was not temperature regulated by the RSV ts-src mutation in RSVtsLA90 mutant cell lines (Table I). The magnitude of the relative abundancies of RNA species in nontransformed and transformed cell lines was variable with all of the other cDNA clones tested and not sufficient to conclude that any systematic regulation was detectable.

Growth Regulation of RNA Levels in Nontransformed Cells

Cellular functions can be regulated by the physiological state of cell growth. For example thymidine kinase activities are high in actively growing cells and low in resting cells [9]. Transformed cells, because they continue to grow and replicate past the cell monolayer, often have been reported to have higher thymidine kinase activities than nontransformed cells [9,10]. The reason for these apparent differences between nontransformed and transformed cell cultures, however, is only related to the replication or growth rate of the cells [9,10]. To avoid these complications in interpretation only growing nontransformed and transformed cell cultures were employed to extract the RNAs used in the experiment shown in Table I. To examine the question of growth regulation of RNA levels in a different experimental manner, serum-arrested 3T3 cell cultures were stimulated into a cell cycle of growth by the addition of 10% fresh fetal calf serum. Entry of these cells into the DNA synthetic stage of the cell cycle (S) was monitored by incorporation of ^{3}H-thymidine into the cells. Entry into the S phase began at 10 hr after addition of fresh serum and DNA synthesis reached a maximal rate at 18 hr after serum addition. Cytoplasmic RNA was extracted from these cell cultures prior to the addition of fresh serum (0 hr) and at 2, 8, 12, and 18

hours after the addition of serum. The RNA was labeled with γ-ATP^{32} and polynucleotide kinase and hybridized to the eleven cDNA clones under study. The experiment was thus designed to examine whether the levels of RNA species complementary to the eleven cDNA clones under study would increase after serum stimulation of growth in nontransformed 3T3 cells.

Four of the cDNA clones (192, 348, 85, and 285) detected a 5–12-fold enhancement of RNA species in 3T3 cells stimulated with serum (Table II). These four cDNA clones were the ones that did not show a consistent differential in RNA levels between nontransformed and transformed cells. The 5–12-fold increase in the abundance of these RNA species in serum-stimulated 3T3 cells was in the range of the differences detected with these cDNAs, when transformed and nontransformed cell lines were compared. Apparently the mRNAs detected by these four cDNA clones are modestly growth regulated. With the cDNA clones A17, 104, 397, 403, 218, B50, and 105 the levels of RNA species in 3T3 cells complementary to these cDNAs were below the level of detection by the dot-blot hybridization procedure employed here (Table II). By contrast, more abundant levels of RNA complementary to these same cDNA clones were always detected in transformed cells (Table I). In spite of this, there was no detectable increase in the RNA species under study here after serum stimulation of 3T3 cells. Clearly, the abundance levels of RNA species measured by these cDNA clones are not stimulated by the addition of serum to the same extent as was observed in a wide variety of transformed cell lines. One can conclude from these experiments that growth control regulation cannot fully account for the enhanced levels of some of these RNA species in the transformed cells compared to nontransformed cells.

DISCUSSION

Starting with eleven cDNA clones that had previously been selected for their ability to detect cellular RNA species at higher levels in SV3T3 cells compared to 3T3 cells, these cDNA clones were employed to measure the abundance levels of cellular RNA species in a wide variety of transformed cell lines. Employing four

TABLE II. Steady State Levels of RNA Species After Serum Stimulation of 3T3 Cells

	Cpm hybridized at time (hr) after serum stimulation				
Clone no.	0	2	8	12	18
A17	0	0	0	0	0
104	0	0	0	0	0
397	0	0	0	0	0
403	0	0	0	0	0
218	0	0	0	0	0
B50	0	0	0	0	0
105	0	0	0	0	0
192	20	96	110	234	72
348	20	40	95	120	95
85	33	163	170	156	149
285	695	2112	1540	2183	3180

Resting 3T3 cells were stimulated by addition of fresh serum. S-phase begins at 10 hr and the rate of DNA synthesis was maximal at 18 hr.

viral transformed cell lines (SV40, polyoma, adenovirus, and RSV) and two nonviral transformed cell lines (Meth A, F9-EC cells) and their nontransformed counterparts, the steady state levels of RNA species complementary to these cDNA clones was measured (Table I). Three of the cDNA clones, A17, 104, and 218 consistently detected 40–100-fold higher levels of cellular RNA in transformed cells than in their nontransformed counterparts. In two cases, A17 and 104, the levels of RNA were regulated in a temperature sensitive fashion by both SV40tsA and RSV ts-src transformed mouse cells. Three cDNA clones, 397, 403 and B50, detected 100-fold or greater RNA levels in SV40-transformed cells, but not in the other transformed cell lines studied here. These RNA species were also regulated in a temperature-dependent fashion in SV40tsA transformed cells. Apparently these RNA species are regulated specifically in SV40-transformed cells.

The differences in abundance levels observed in nontransformed cells compared with transformed cells can not be ascribed to the growth control regulation of these RNA species. Cellular RNA's complementary to these cDNA clones were not detected in actively growing nontransformed cells nor in serum-stimulated 3T3 nontransformed cells. The abundance levels of these RNAs in transformed cells was higher than in growing nontransformed cells. Some of the cDNA clones tested (192, 348, 85, 285) detected cellular RNA species that were modestly stimulated in their levels (5–12-fold) by serum stimulation of growth arrested 3T3 cells. These cDNA clones detected RNA species only moderately regulated, if at all, in transformed versus nontransformed cell lines. These conclusions are summarized in Table III.

The dot-blot hybridization procedure employed here [8] is useful to screen large numbers of RNA preparations from different cell lines. The use of RNA, labeled with γ-ATP^{32} and polynucleotide kinase, measures the steady state levels of these RNA species derived from the cell lines under study. There are, however, several drawbacks to this experimental procedure. The level of detection of low abundance RNA species in a cell line is not high and this technique is as much as 100-fold less sensitive than the Northern gel hybridization procedure [11] using nick-translated DNA at high specific activities [12]. This has lead to the failure to detect some RNAs (Tables I and II) in nontransformed cells and therefore only approximate quantitation. This drawback, however, does not change the qualitative conclusions of this work for two reasons. First, Northern gel hybridization, using high specific activity DNA as a probe, has been employed to confirm some of these results [1]. Second, in all cases where no RNA was detected in nontransformed cells, detectable levels of RNA were

TABLE III. Summary of the Properties of RNA Levels Detected With the cDNA Clones

Clone no.	Properties of the RNA species
A17, 104, 218	RNAs are at enhanced levels (40–100×) in many transformed cell lines compared to nontransformed cells. A17 and 104 RNAs are ts-regulated in SV40 tsA and RSV ts-src cells.
397, 403, B50	Enhanced RNA levels (100×) in SV40-transformed cell lines but not in other transformed cell lines.
192, 348, 85, 285	Modestly growth regulated RNAs (5–12×) with no consistent or major differences in nontransformed and transformed cell lines.

found in transformed cells using dot-blot hybridization (Table I). These results therefore demonstrate the higher abundance levels of these RNA species in transformed cells, but this type of result does not permit accurate quantitation. Thus only abundance differences of RNA levels greater than 50–100-fold are clearly significant (Table I).

Secondly, total cytoplasmic RNA preparations were employed in all of the studies, to measure RNA levels in transformed and nontransformed cells. In several cases these cDNA clones detect multiple RNA species in the cytoplasm of transformed and nontransformed cells [1]. Indeed, in some cases, such as clone 85, there is a differential regulation in the levels of these multiple RNA species when transformed and nontransformed cells are compared by Northern gel hybridization [1]. Clone 85 detects a very abundant but poorly regulated RNA transcript and so no regulation is detected between normal and transformed cell lines when total cytoplasmic RNA is analyzed. In fact, clone 85 cDNA does detect different low abundance RNA species that are present in SV3T3 cells but not 3T3 cells [1]. Furthermore, infection of 3T3 cells with SV40 stimulates the levels of clone 85 RNAs [1] indicating that the population of clone 85 RNAs are regulated under certain circumstances. Because of these complexities it may be premature to make too many conclusions about cDNA clones that were not shown to be regulated in transformed cells by this type of study (dot-blot hybridization).

The results of the experiments presented here suggest that some cellular RNA species are detected in enhanced levels in all transformed cells independent of the transforming agent. These enhanced levels cannot be totally ascribed to cellular growth regulation. These transformation-related RNA species could provide new insights into the events common to the transformation process. Further experimentation will be required to confirm this notion quantitatively and to elucidate the mechanism that results in enhanced abundance levels of some cellular transcripts in transformed cells.

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