Volume 14 Number 18 1986 Nucleic Acids Research

Alteration of cellular gene expression in adenovirus transformed cells by post-transcriptional mechanisms

Hung-Teh Kao and Joseph R.Nevins

Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10021, USA

Received 5 June 1986; Revised and Accepted 8 August 1986

ABSTRACT

We have isolated cDNA clones complementary to human mRNAs that are expressed at elevated levels in 293 cells, adenovirus-transformed human embryonic kidney cells, as compared to a normal counterpart of this cell line. Approximately 200 clones out of 100,000 that were screened were positive; 40 of these were isolated, of which 31 were determined to be unique and were further characterized. Each clone detected a mRNA that was 5 to 50 times more abundant in 293 cells than in the non-transformed HEK cell line. For several of these transcripts, the elevated expression appeared to be a function of transformation since they were also high in other human tumor cell lines. Strikingly, we have found that post-transcriptional control is largely responsible for the regulation of the abundance of these mRNAs.

INTRODUCTION

The phenotype that characterizes a particular cell type is in part the result of the expression of a specific set of genes as well as the unique combinatorial interactions of these gene products. Two issues arise as to the nature of cellular phenotypic differences. First, what are the gene products that characterize a particular cell and what can this information tell us about the contributions of specific gene products to the ultimate phenotype of the cell. Second, what is the basis for the unique expression of particular genes in one cell type versus another. We have explored these questions through the isolation and analysis of genes expressed at increased levels in cells transformed by adenovirus.

MATERIALS AND METHODS

Cells

The growth and maintenance of the human 293 cell line(1) have been described previously. A human embryonic kidney (HEK)

© IRL Press Limited, Oxford, England.

cell line (diploid fibroblasts of limited life span) was obtained from Flow Laboratories. All cells were maintained in DME with 10% fetal calf serum with the exception of HL60 which was grown in RPMI. All cells were sub-confluent when used for analysis.

Preparation and Screening of λgt10 cDNA Library

cDNA was synthesized from 293 cell poly A+ RNA and inserted into the Eco RI site of λgt10 (2). For the preparation of a 293 specific probe, ^{32}P cDNA was prepared from 293 polyA+ RNA and then hybridized to a large excess of HEK RNA. Hybridized sequences were removed by two passages through hydroxyapatite. The resulting single strand ^{32}P cDNA was used as a hybridization probe.

Northern Analysis

Total cell RNA was prepared by guanidinium isothiocyanate lysis and cesium chloride fractionation (3). Equal aliquots (50 μg) of various samples were analyzed in agarose-formaldehyde gels as described before (4,5).

Isolated Nuclei Transcription

The assay of transcription rates in isolated nuclei was performed as described (6).

RESULTS

We have sought to identify sequences present in the mRNA populations of 293 cells, an adenovirus transformed human embryonic kidney cell line (1), that were not present in mRNA of a normal human embryonic kidney (HEK) cell line. Although the exact original parent of the 293 cell line is not available, these HEK cells are nevertheless similar as they were drived from the same tissue source. A ^{32}P-labeled cDNA copy of poly A+ 293 cell mRNA was prepared and hybridized to a large excess of RNA from HEK cells, and passed through a hydroxyapatite column so as to remove hybridized sequences and thus eliminate those labeled cDNAs common to mRNAs in both cell lines. The resulting single strand ^{32}P-labeled cDNA should therefore be specific for RNAs expressed in the 293 cells. In fact, there was very little material remaining that was capable of hybridization to HEK RNA in a Northern (data not shown). The unhybridized 293 cell specific ^{32}P-labeled cDNA

was then used as a hybridization probe to screen a lambda gt10 library of 293 cell cDNA (2). The screening of 100,000 plaques with such a probe yielded 200 to 300 positives or roughly 0.2% to 0.3% of the total. Approximately, 40 of these were isolated, re-screened, and those that remained clearly positive were isolated and grown for further analysis. Most of these were then sub-cloned into the plasmid vector pUR250 (6a). To determine the extent to which the isolated clones represented unique sequences, a series of cross hybridization studies were performed. Of the initial 40 clones that were isolated, 31 of these appeared to be unique. Since we did isolate the same sequence in several cases, we anticipate that we are close to the limit of the abundant, differentially expressed transcripts.

To determine if indeed the screening procedure was effective in selecting 293 cell specific sequences as well as to further characterize the mRNAs, we utilized the various recombinant cDNAs as probes for Northern blots. Total cell RNA from 293 cells and HEK cells (50 μg of each) was fractionated in agarose-formaldehyde gels, transferred to nitrocellulose and then probed with nick-translated cloned DNAs. Examples of such analyses are shown in Figure 1. Each of the selected clones detected an mRNA that was anywhere from 5 to 50 fold more abundant in the total RNA from 293 cells than in HEK cells. Furthermore, the same results were obtained by analyzing equal amounts of poly A+ RNA from the two cell types (data not shown). In most cases, the RNA was expressed in the HEK cells but was greatly increased in abundance in the 293 cells. In addition, most of the clones detected a single mRNA species in either the 293 cells or the HEK cells although the clone pN35 hybridized to two RNA species of .35 kb and 0.6 kb in length. Finally, as a control we have employed a chicken actin cDNA clone for hybridization to the same RNAs; in contrast to the various selected cDNA clones the actin mRNA appears to be slightly more abundant in the HEK cells than in 293 cells.

Clearly, these various mRNAs are expressed at quite different levels in 293 cells and HEK cells. However, given

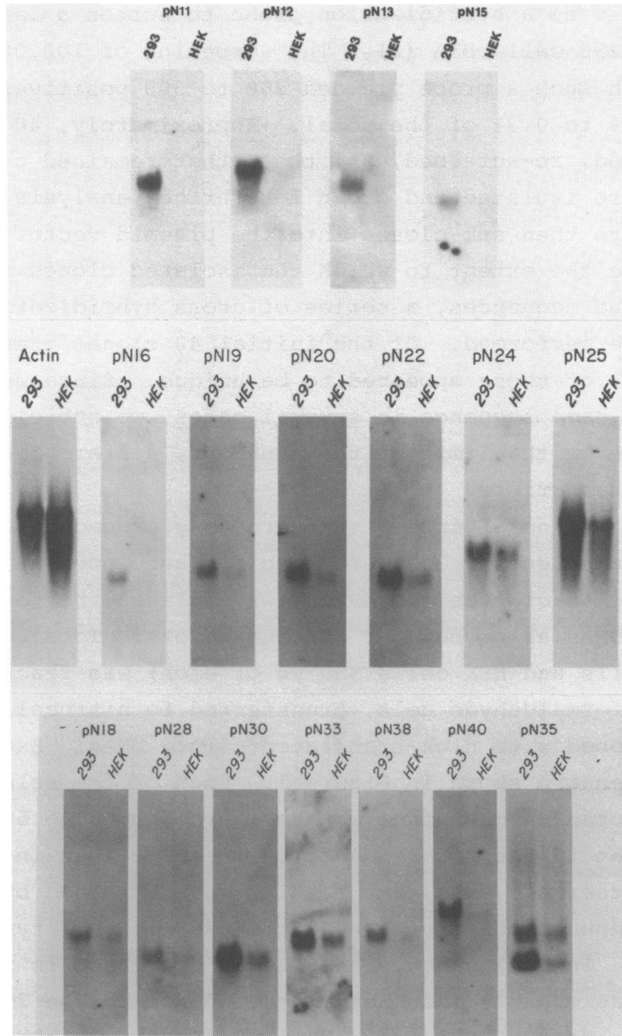

Figure 1: Northern analysis of RNA from HEK and 293 cells probed with 293 cell specific cDNAs. Total cellular RNA was prepared from HEK cells and 293 cells. Aliquots of 50 µg were subjected to Northern analysis and hybridization to the various 293 specific cDNAs.

the distant relationship of the two cell lines, we could not be certain of the basis for this increased expression. As one approach to this question, we determined if the increased expression was a function of transformation in general. RNA was

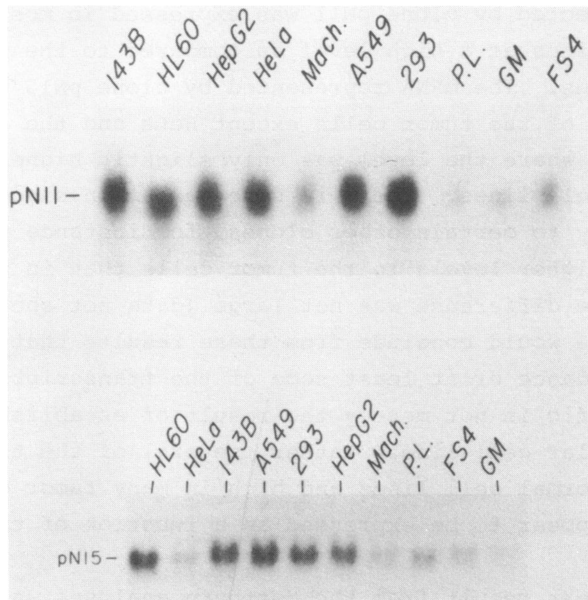

Figure 2: Northern analysis of RNA from human tumor cell lines and normal cell lines probed with 293 specific cDNAs. Total cell RNA was prepared from various human cell lines as well as from human peripheral lymphocytes (P.L.). Equal amounts of each (50 μg) was subjected to Northern analysis and hybridization with various 293-specific cDNAs. The origin of the various tumor cell lines is described in the text. The FS4 cell line derives from neonatal foreskin fibroblasts and are normal diploid cells. The GM2504 cell line is similar to FS4 but was derived from a chromosome 21 trisomy.

prepared from a number of human cell lines derived from tumor sources or transformed in culture. These include a promyelocytic leukemia (HL60), a lung carcinoma (A549), a cervical carcinoma (HeLa), a hepatoma (HepG2), an osteosarcoma transformed in culture by Kirstein sarcoma virus (143B), and an astrocytoma (Machicao). In addition, RNA was prepared from what were considered to be three normal, non-transformed cell types: two diploid fibroblast cell lines (FS4 and GM) as well as human peripheral lymphocytes. Equal amounts of each RNA sample were analyzed in Northern blots and probed with several cDNAs (Figure 2). Although we have as yet only tested a limited number of the clones, of those we have tested, most show a transformation-specific pattern of expression. For example,

the mRNA detected by clone pN11 was expressed in most of the tumor cell lines at a high level as compared to the three normal cell lines. The mRNA represented by clone pN15 was abundant in most of the tumor cells except HeLa and the astrocytoma (Mach.) where the level was only slightly higher than in the normal cell lines. There is some variation since the mRNA complementary to certain other clones, for instance pN30, were present at higher levels in the tumor cells than in the normal cells but the difference was not large (data not shown). Therefore, we would conclude from these results that the increased abundance of at least some of the transcripts isolated as 293 specific is not merely the result of establishment of this particular cell line. Rather, several of the transcripts are low in normal cell lines and high in many tumor cell lines. Thus, they appear to be expressed as a function of transformation.

The clear result from the Northern analyses was the large difference in abundance between 293 cells and HEK cells for most of the transcripts. As this represents a potential set of coordinately controlled genes, the mechanism of such control is of importance, particularly when it relates to transformation. To determine the level at which the abundance of these various mRNAs was regulated, we employed isolated nuclei to measure transcription rates. Nuclei were prepared from HEK cells and 293 cells and incubated _in vitro_ with ^{32}P-UTP. The labeled RNA was then hybridized to filters bearing most of the 293 cell specific clones as well as certain other DNAs serving as controls. The results of one such experiment are shown in Figure 3. The most striking result evident from this experiment, after comparison to the data of Figure 1, was that the alteration of cytoplasmic abundance of many of the transcripts could not be accounted for by their rate of nuclear transcription. For instance, the mRNA complementary to clone pN15 was at least 50-fold more abundant in 293 cells than in HEK cells (Figure 1). Yet, the rate of transcription of this gene was no greater in 293 cells than in HEK cells. The same was true for many of the other 293 cell-specific transcripts, although in some cases there may have been a

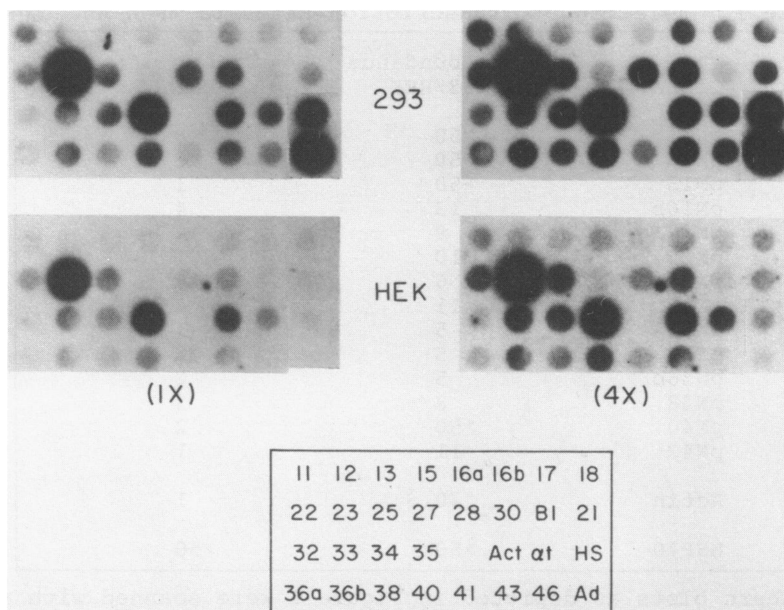

Figure 3: Transcription rate measurements of 293 cell specific genes in HEK cells and 293 cells. Nuclei were prepared from 5 x 10^7 293 cells and 5 x 10^7 HEK cells and incubated *in vitro* with ^{32}P-UTP. Equal amounts (CPM) of labeled nuclear RNA were hybridized to duplicate filters bearing the indicated cDNAs. After hybridization, the filters were washed, treated with RNase, and used to expose X-ray film. As indicated, the exposures on the right were 4 times longer than those on the left. At the bottom is shown a key indicating the positions of the various cDNAs on the filters. Bl = blank; αt = alpha tubulin; HS = hsp70; Ad = adenovirus E1A + E1B.

small transcriptional increase that contributed to the overall mRNA increase. For example, pN22, 25 and 42 were transcribed at the same rate in the two cell lines whereas pN11 was transcribed at an 8-fold higher rate in 293 cells than HEK. Even this 8-fold transcription increase, however, could not account for the increase in mRNA abundance, in this case greater than 50-fold. In only one case did the transcription rate difference begin to approach and account for the steady state mRNA difference. The transcription rate of the gene complementary to clone pN28 was reproducibly higher in 293 cells than in HEK cells and the difference was roughly equivalent to the diff-

Table 1. Relation of Transcription Rates to mRNA Abundance

Clone	mRNA Abundance[1] 293/HEK	Trancription[2] 293/HEK
pN11	>50	8
pN13	>50	2
pN15	>50	1
pN16b	13	4
pN22	9	1
pN25	10	1
pN28	6	14
pN30	11	4
pN33	5	1
pN34	5	2
pN36b	5	2
pN38	8	2
pN40	>50	2
pN42	11	1
Actin	0.3	1
HSP70	>50	>50

1 Northern blots as depicted in Figure 1 were scanned with a densitometer and the ratio of the 293 level versus the HEK level was calculated.

2 Transcription measurements as shown in Figure 3 were scanned with a densitometer, correcting for background hybridization, and the relative values of 293 versus HEK were calculated. Data is presented for those clones that yielded a signal above background and for which data from Northerns was available. For all clones except 13, 15, 25, 34, 38 and 42, the values are the average of two independent measurements. The clones pN23 and pN35 appear to contain repetititve sequences as judged from the very high rate of transcription.

erence in mRNA abundance. Finally, we have previously isolated a clone to the hsp70 mRNA and demonstrated a greatly increased abundance in 293 cells as compared to HEK (5). Transcription analysis of this gene revealed that, as with the pN28 gene, the regulation of the abundance of the hsp70 RNA between 293 cells and HEK cells was also transcriptional. These results are summarized in Table 1. Therefore, regardless of the reason for the change in the abundance of these mRNAs, the clear result from the transcriptional analyses is that the majority of the control is post-transcriptional.

DISCUSSION

Our approach in these studies has been similar to previous work that has sought to isolate and characterize the set of genes uniquely expressed as a result of oncogenic transformation (7,8). However, we have investigated the level of control for the altered expression of these genes. Somewhat surprisingly, our results suggest that post-transcriptional control is a major factor in the RNA differences in these cells and therefore that regulatory interactions in this instance presumably must involve protein-RNA recognition rather than protein-DNA. Certainly, there are other examples of the control of gene expression through post-transcriptional means. The control of the DHFR gene in relation to cell growth appears to be largely due to post-transcriptional control (9,10), and may involve poly A site selection (11), although there may also be transcriptional effects as well (12). Furthermore, the hormone-induced activation of the α1-acid glycoprotein gene involves a post-transcriptional nuclear RNA processing event (13). And, there are examples of tissue-specific and differentiation specific control of gene expression through the control of poly A site selection (14, 15). The distinction made by the data presented here is in the scope of the effect; an entire set of genes are post-transcriptionally regulated. Possibly a relevant example comes from recent experiments studying the control of certain mouse liver specific genes in primary liver cultures, mouse hepatoma cells, and variants of these hepatomas that have lost and then regained the expression of liver specific functions (16).

The main point from our studies is that the alteration of control of expression of these genes is not due solely to a mechanism of transcriptional control involving DNA sequence recognition. The exact nature of the control that is responsible for such differences is not clear; obviously, RNA processing, nuclear-cytoplasmic transport and cytoplasmic stability are all reasonable possibilities. Interestingly, recent experiments point to a role for the adenovirus E1B 55K protein in the post-transcriptional metabolism of both viral

and cellular mRNA. In the absence of this gene product there is a reduced accumulation of late viral mRNAs despite the fact that there is no decrease in transcription (17,18). Furthermore, there is no shut-off of mRNA transport or translation as normally occurs late in viral infection. Whether the increased accumulation of these cellular transcripts in 293 cells is the result of the action of the E1B gene is not clear. We have found that the abundance of these transcripts is not increased by lytic infection of the HEK cells with Ad5. However, this does not mean that under different circumstances (for instance, the absence of other viral gene products that would be present in lytic infection) that the E1B protein might have such an effect. As an example, in a lytic infection there is an association of the 55K E1B protein with a product of the viral E4 gene (19) whereas in transformed cells the 55K protein associates with the p53 tumor antigen (20). Possibly such associations modify the action of the 55K protein with respect to substrates, a modification that may indeed be important for the transformation event.

ACKNOWLEDGEMENTS

We thank Wai Wong for his superb technical assistance. This work was supported by a grant from the NIH (GM26765).

REFERENCES

1. Graham,F.L., Smiley,J., Russell,W.C. and Nairn,R.(1977). J.Gen.Virol. 36, 59-72.
2. Huynh,T.V., Young,R.A. and Davis,R.W.(1984) In Gover, D. (ed), DNA Cloning Techniques: A Practical Approach, IRL Press, Oxford.
3. Chirgwin,J.M., Prysbyla,A.E., MacDonald,R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
4. Thomas, P.S. (1980) Proc. Nat'l. Acad. Sci. USA 77, 5201-5205.
5. Kao, H.-T. and Nevins, J.R. (1983) Mol. Cell. Biol. 3, 2058-2065.
6. Clayton,D.F. and Darnell,J.E. (1983) Mol. Cell. Biol. 3, 1552-1561.
6a. Ruther,U. (1982) Nucl. Acids Res. 10, 5765-5772.
7. Schutzbank,T., Robinson, R., Oren,M. and Levine,A.J. (1982) Cell 30, 481-490.
8. Scott,M.R.R., Westphal,K.-H. and Rigby,P.W.J. (1983) Cell 34, 557-567.
9. Leys,E.J., Crouse,G.F. and Kellems,R.E. (1984) J. Cell. Biol. 99, 180-187.

10. Leys,E.J. and Kellems,R.E. (1981) Mol. Cell. Biol. 1, 961-971.
11. Kaufman,R.J. and Sharp,P.A. (1983) Mol. Cell. Biol. 3, 1598-1608.
12. Santiago,C., Collins,M. and Johnson,L.F. (1984) J. Cell. Physiol. 118, 79-86.
13. Vannice,J.L., Taylor,J.M. and Ringold,G.M. (1984) Proc. Nat'l. Acad. Sci. USA 81, 4241-4245.
14. Mather,E.L., Nelson,K.J., Haimovich,J. and Perry,R.P. (1984) Cell 36, 329-338.
15. Amara,S.B., Evans,R.M. and Rosenfeld,M.G. (1984) Mol. Cell. Biol. 4, 2151-2160.
16. Clayton,D.F., Weiss,M. and Darnell,J.E. (1985) Mol. Cell. Biol. 5, 2633-2641.
17. Babiss,L.E. and Ginsberg,H.S. (1984) J. Virol. 50, 202-212.
18. Babiss,L.E., Ginsberg,H.S. and Darnell,J.E. (1985) Mol. Cell. Biol. 5, 2552-2558.
19. Sarnow,P., Hearing,P., Anderson,C.W., Halbert,D.N., Shenk, T. and Levine,A.J. (1983) J. Virol. 49, 692-700.
20. Sarnow,P., Ho,Y.S., Williams,J. and Levine,A.J. (1982) Cell 28, 387-394.