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Isolation of Cellular Genes Differentially Expressed in Mouse NIH 3T3 Cells and a Simian Virus 40-Transformed Derivative: Growth-Specific Expression of VL30 Genes

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We constructed and screened a cDNA libary made from simian virus 40 (SV40)-transformed NIH 3T3 cells, and we isolated cDNAs representing genes that are differentially expressed between the parental cell and its SV40-transformed derivative. We found only a small number of cDNAs representing such genes. Two isolated cDNA clones represented RNAs expressed at elevated levels in the transformed cell line in a manner relatively independent of growth conditions. The expression of two other cDNAs was growth specific because transformed cells and nonconfluent parental cells contained higher levels of the homologous RNAs than did confluent, contact-inhibited parental cells. Another cDNA was well expressed in confluent parental and confluent transformed cells, but not in nonconfluent cells. The expression of some of these cDNAs varied strikingly in different mouse cell lines. Thus the genotype or histories of different cell lines can also affect the expression of certain genes. Interestingly, the only cDNA isolated that was expressed exclusively in the transformed cell was from an SV40 message. We focused on a growth-specific cDNA which we show is derived from a mouse endogenous retrovirus-like family called VL30. We sequenced the 3′ long terminal repeat (LTR) of this transcriptionally active VL30 gene. This LTR has good homology with other VL30 LTR sequences, but differences occur, particularly upstream of the VL30 promoter. We found that VL30 gene expression varied in different mouse cell lines such that C3H cell lines had very low levels of VL30 transcripts relative to NIH 3T3 cell lines. However, Southern analysis showed that both cell lines had about the same number of VL30 genes homologous to our probe and that the position of the majority of these genes was conserved. We discuss possible explanations for this difference in VL30 expression.

The simian virus 40 (SV40)-encoded large T antigen is a complex multifunctional protein which is required for both the establishment and maintenance of the transformed phenotype (6, 22, 24, 29, 42, 44). While many theories have been promulgated to account for this transformation function, an area that has only recently been explored concerns the ability of the protein to alter the transcriptional or other nuclear events concerned with gene expression.

Recent experiments have clearly shown that T antigen plays a role in the transactivation of SV40 late gene expression (5, 18). This transactivation may be, at least in part, an indirect effect of T antigen (18; P. Robbins and M. Botchan, unpublished data). In this regard, we and others (37, 38) have been interested in the isolation of cellular genes whose expression might be affected by SV40. It is unlikely that all such genes will lead directly to functions involved in transformation. They may, however, provide other systems to study the mechanisms through which the virus can alter gene expression.

In this report we describe the isolation and characterization of cDNAs representing genes that are differentially expressed between NIH 3T3 cells and an SV40-transformed derivative, SVLTR1 (23). To isolate such genes, we constructed a cDNA library representing the polyadenylated [poly(A)$^+$)] mRNA population of the SV40-transformed cell line. We wished to study both transformation- and growth-specific changes in gene expression and therefore screened our library with probes that would allow us to isolate both types of genes. We found only a small number of cDNAs representing genes that are differentially expressed. Among these cDNAs we found examples of both transformation- and growth-specific expression.

We have begun to characterize some of these differentially expressed cDNAs. In another study we focused on the characterization of one such SV40 transformation-induced gene and showed that the transcription unit is the product of a polymerase III gene (40). In the second part of this report we focus on a cDNA clone whose expression is growth specific. We show that this cDNA clone is derived from a mouse VL30 (virus-like 30S RNA) transcript (2). Mouse VL30 elements are a dispersed class of moderately repetitive 5- to 6-kilobase-pair DNA sequences with structural features similar to those of integrated forms of retroviruses and certain classes of transposable genetic elements (20). It is estimated that about 100 to 200 VL30 elements exist in many mouse cell lines (19, 21). Recently, solo VL30 long terminal repeats (LTRs) have also been isolated (35). A growing body of evidence indicates that murine transposable elements, like transposable elements in other systems, are able to have profound effects on the expression of other cellular genes. Thus insertion of intracisternal A-particle elements (another repeated, retrovirus-like gene family) within cellular genes resulted in one case in a defective immunoglobulin light-chain gene (26), while in another case, intracisternal A-particle insertion resulted in increased expression of the c-*mos* gene (25, 32).

MATERIALS AND METHODS

Cell culture and RNA isolation. Cells were grown in Dulbecco modified minimal essential medium containing 10%

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calf serum. The isolation of cytoplasmic RNA and Northern blot analysis was as previously described (40). Poly(A^+) RNA was isolated by two binding-elution cycles on oligo(dT) cellulose type T-2 from Collaborative Research, Inc., as described by the supplier.

cDNA library construction and screening. Four micrograms of poly(A^+) RNA was used in the construction of the SVLTR1 cDNA library, essentially as described by Nathan and Hogness (30), to give 2.8×10^5 independent phage with cDNA inserts. These phage were amplified, and the resulting phage lysate was stored in 7% dimethyl sulfoxide at −70°C. Duplicate nitrocellulose filters were made by standard procedures (28) from large plates (150 by 15 mm) containing ≈500 plaques each. ^{32}P-labeled single-stranded cDNA probes (≥10^8 cpm/μg) from confluent, contact-inhibited 3T3 cells and from nonconfluent actively growing SVLTR1 cells were prepared as described by Davis et al. (12). One set of filters was hybridized with 5×10^7 cpm of the SVLTR1 cDNA probe, while the duplicate set of filters was hybridized with 5×10^7 cpm of the 3T3 cDNA probe. Prehybridization and hybridization conditions were as described elsewhere (28). Filters were washed extensively and exposed to X-ray film by standard procedures (28). Plaques that showed differential expression were picked and rescreened. Plaques that hybridized more with either pobe were picked and further characterized. Banded DNA was prepared from phage that consistently showed differential expression with confluent plate lysates and CsCl step gradients (28). The *Eco*RI cDNA inserts were subcloned into the unique *Eco*RI site of pBR322 by standard procedures (28). Plasmid DNA was prepared by the procedure of Birnboim and Doly (4), banded twice in $CsCl_2$, and dialyzed into 10 mM hydrochloride (pH 7.5)–1 mM EDTA.

Isolation and analysis of genomic DNA. Genomic DNAs from the NIH 3T3 and C3H cell lines were prepared by standard procedures (28) based on a modification of the method of Thomas et al. (45). Southern blot analyses of these DNAs were carried out by standard procedures (28). Genomic DNA (10 μg) was digested with *Eco*RI and fractionated by electrophoresis through 1% agarose gels.

DNA sequencing. DNA sequence analysis was performed by the dideoxy chain termination method of Sanger et al. (36) with the modifications suggested by Biggen et al. (3). The *Bgl*II-*Eco*RI, *Bgl*II-*Xba*I, and *Eco*RI-*Xba*I fragments were cloned in both orientations into the M13 vectors TG130 and TG131 from Amersham Corp.

RESULTS

Isolation of cDNAs representing differentially expressed genes. To isolate differentially expressed genes, we constructed a cDNA library representing the poly(A^+) mRNA population of an SV40-transformed mouse NIH 3T3 cell line, SVLTR1 (23), by using a λ cloning vector. The construction of the library and subsequent screening are described in Materials and Methods. The library contained approximately 2.8×10^5 independent cDNA clones. For moderately expressed RNAs, the library appeared to be representative, as the fraction of cDNA clones containing T-antigen-related transcripts is what we would predict from the known expression levels of the SV40 early messages. We probed duplicate filters of the cDNA library with labeled cDNAs made from poly(A^+) cytoplasmic RNA extracted from either rapidly growing transformed cells or contact-inhibited monolayers of parental NIH 3T3 cells. Using these probes, we expected to find the following two broad classes of differentially expressed genes: (i) those cDNAs whose levels of expression are increased or reduced specifically in SV40-transformed cells, i.e., transformation specific; and (ii) those cDNAs whose expression simply reflects the transition from growing to resting states. From an initial screen of 10,000 independent phage, only a small number of plaques (0.1%) were found that showed indications of inserts representing differentially expressed RNAs.

Analysis of RNAs homologous to differentially expressed cDNAs. Those cDNAs that consistently showed differential expression were isolated, subcloned into pBR322, and used as probes in Northern blot analyses as described in the legend of Fig. 1. This figure shows the cytoplasmic transcriptional levels of some of our differentially expressed cDNAs in the parental NIH 3T3 mouse cells and the SV40-transformed derivative. For both cell lines, cytoplasmic

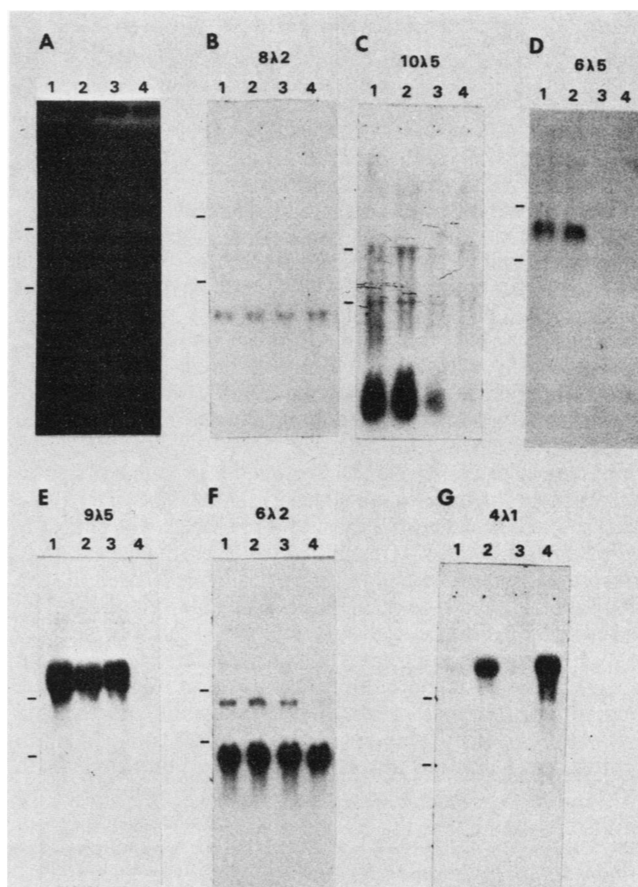

FIG. 1. Northern blot analysis of transcripts with homology to differentially expressed cDNA clones. The lanes within each panel contain RNAs from cells grown to different densities. Each lane contains 20 μg of cytoplasmic RNA. For each panel the lanes are: (1) SVLTR1, cell density of 2.3×10^6; (2) SVLTR1, cell density of 18×10^6; (3) NIH 3T3, cell density of 2.7×10^6; (4) NIH 3T3, cell density of 5.5×10^6. Lanes 1 and 3 represent nonconfluent cells, and lanes 2 and 4 represent confluent cells. (A) Acridine orange staining of an agarose-formaldehyde gel showing position and relative amounts of 28S and 18S rRNAs in each lane. The remaining panels, B through G, are Northern blots probed with the following cDNA probes: (B) 8λ2 (a nondifferentially expressed cDNA clone), (C) 10λ5, (D) 6λ5, (E) 9λ5, (F) 6λ2, and (G) 4λ1. The positions of the 28S and 18S rRNAs are represented by lines on the left of each panel. In overexposed autoradiograms of total cytoplasmic RNA, some hybridization to both 18s and 28s RNAs is detectable with some of our probes.

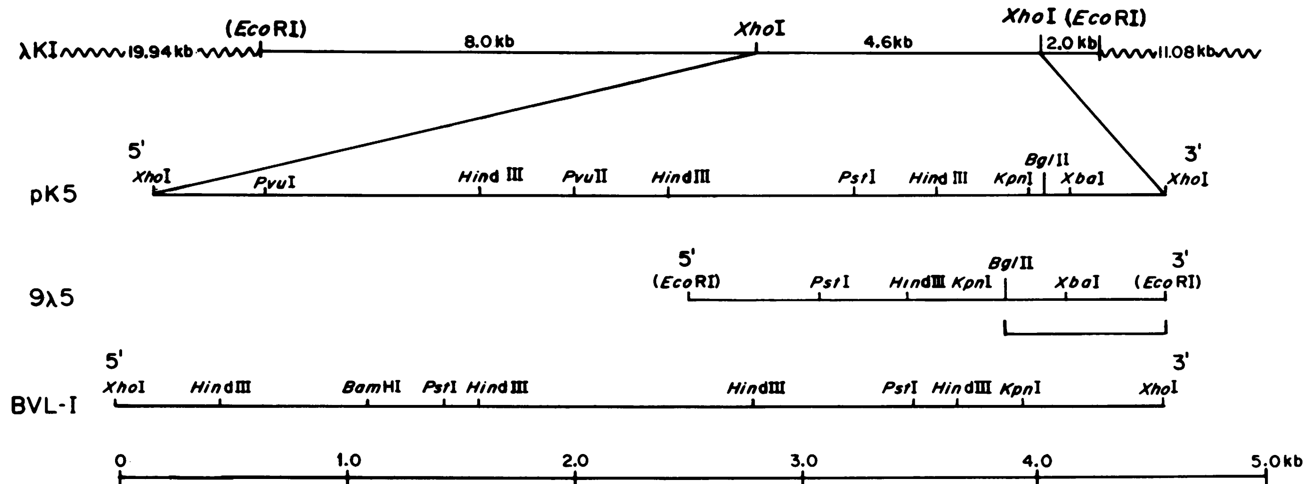

FIG. 2. Comparison of restriction enzyme maps of various VL30 genomic and cDNA clones. λK1 is a Charon 4A clone (and contains a 14.6-kilobase-pair genomic insert) which we isolated from a mouse BALB/c genomic library (43). The 4.6-kilobase-pair *Xho*I fragment of λK1 was cloned into the *Sal*I site of pBR322 to give pK5. The cDNA clone 9λ5 is described in the text. BVL-1 is another VL30 genomic clone (15).

RNA was extracted from either growing nonconfluent cells, (Fig. 1, lanes 1 and 3) or from cells that had been at confluency for 2 days (lanes 2 and 4). In the case of parental cell lines, cells in such a confluent monolayer are contact inhibited for cell division. Equal amounts of cytoplasmic RNA were used for each time point as shown by acridine orange staining of the gels and measurement of the 18S and 28S RNAs (Fig. 1A) or by probing of a blot from such a gel with a cDNA clone representing a uniformly expressed message (Fig. 1B).

We characterized five cDNA clones that showed significant differential expression. These cDNAs fall into three classes. The expression of one type of cDNA was transformation specific (Fig. 1C and D), as the transcripts they detected were more abundant in the transformed cell than in the parental cell, irrespective of growth conditions. The expression of another type of cDNA was growth specific (Fig. 1E and F), as the transcripts they deteced were present at high levels in both the transformed cell and the nonconfluent parental cells, but at substantially reduced levels in the contact-inhibited parental cell. It is important to point out here that our initial screen of the library was with cDNA probes from RNA extracted from cells showing patterns as illustrated in lanes 1 and 4 on the gels (Fig. 1). Thus 10λ5, 6λ5, and 9λ5 all appeared as induced genes in the initial screen. 6λ2 appeared only marginally induced, presumably because of expression of the second-higher-molecular-weight band shown in Fig. 1F. We also isolated cDNAs whose expression cannot be considered transformation specific or growth specific. Thus, the cDNA clone 4λ1 showed an unusual pattern of expression. The large ≥30S transcript detected by this clone was abundant in both the parental and transformed cells that had been at confluency for 2 days (Fig. 1G). However, in the nonconfluent cells of both parental and transformed type, this transcript was present at low, barely detectable levels.

We characterized some of the cDNA clones shown in Fig. 1. We now know that the cDNA clone 10λ5 contains a B2 repeat and that the small heterogenously sized, cytoplasmic RNAs detected by 10λ5 are polymerase III transcripts encoded by the B2 repeats (40). The cDNA clone 6λ5 turns out to be derived from an SV40 early message. Interestingly, this was the only cDNA isolated in our screen that was exclusively expressed in the transformed cell. The isolation of an SV40 cDNA through this blind screen reinforced our confidence in the technical aspects of the experiments with regard to both the construction of the library and the subsequent screening.

Clone 9λ5 was derived from a VL30 transcript. Clone 9λ5 has homology to a 30S transcript whose expression is growth specific (Fig. 1E). As the large 30S transcript detected by 9λ5 was in the size range to be expected for an endogenous retrovirus, we first asked if the sequences within the cDNA hybridized by Southern analysis to known endogenous retrovirus-like elements. By this criterion, both 9λ5 and a related cDNA clone, 14λ5, also obtained in the library screen (data not shown), appeared to be derived from the transcription product of a member of the mouse endogenous retrovirus-like family called VL30 (2). Sequence analysis of part of 9λ5 confirmed this opinion (see below). It is worth mentioning that in an earlier attempt in this laboratory to isolate differentially expressed genes, we attempted to prepare a transformed-mRNA-specific probe by annealing labeled cDNAs representative of transformed cell mRNA with an excess of mRNA extracted from the parental cells. This differential probe was then used to screen a mouse genomic library. The one fully characterized genomic clone that we isolated, λK1, is also a member of the VL30 family (Fig. 2). In Fig. 2 we compare the restriction map of 9λ5 to the maps of pK5, a subcone of λK1, and BVL-1 (15), another genomic VL30 clone. By restriction mapping criteria, the cDNA clone 9λ5 is more closely related to the VL30 element in pK5 than to that in BVL-1.

Sequence of the 3′ LTR of a VL30 transcript. To confirm the conclusions described above, we sequenced a part of 9λ5. A number of VL30 LTRs have now been isolated and analyzed, and some have been sequenced (15, 17, 31, 35). These VL30 LTRs have all the characteristics of retrovirus LTRs, including the structure 5′ U3-R-U5 3′ (31). There is, however, considerable sequence variation among the VL30 LTR sequences. This variation may be important, since it appears that only a few members of the VL30 gene family are transcriptionally active (8, 19). However, with one exception (31), the VL30 LTRs sequenced to date are

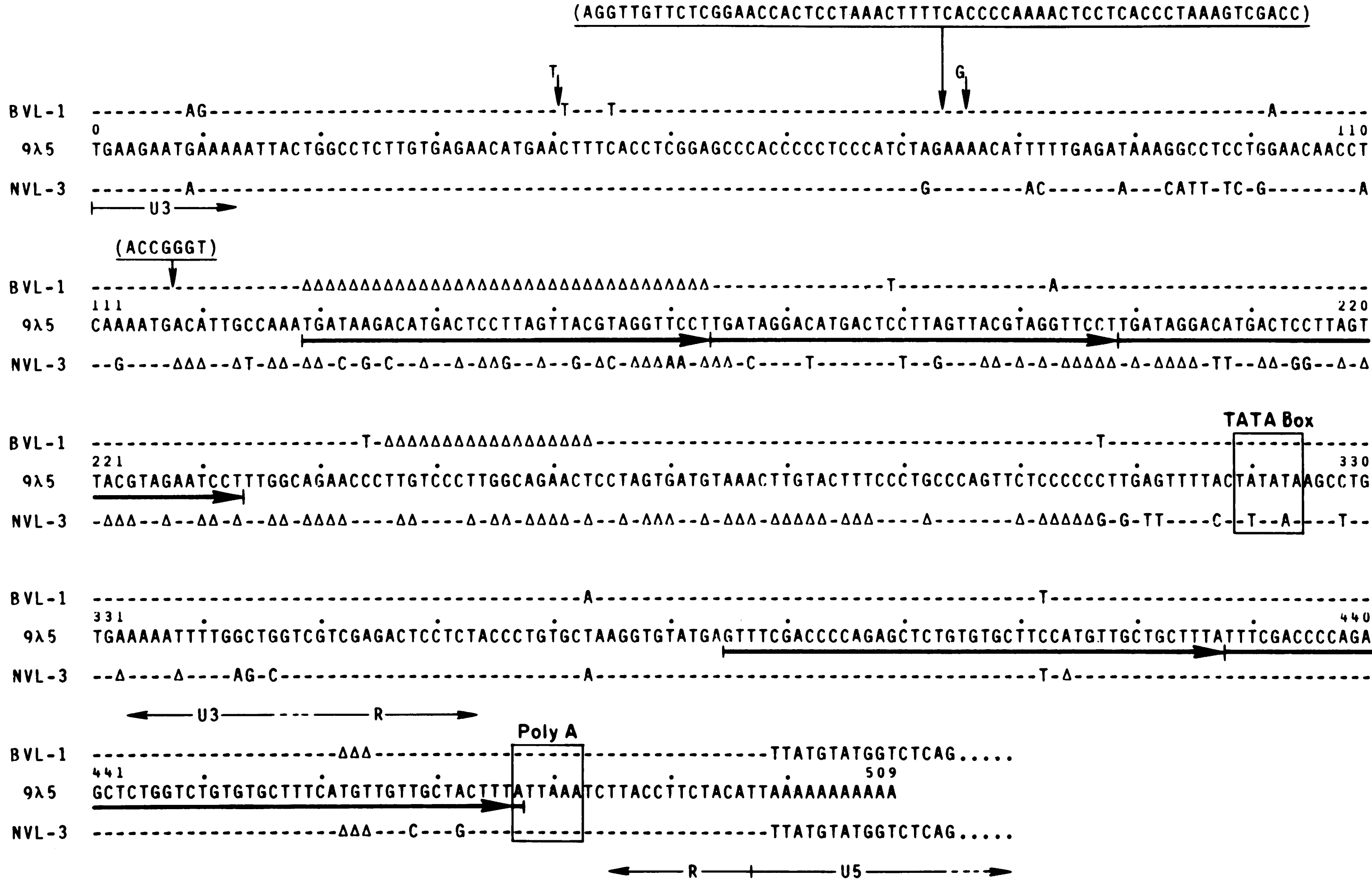

FIG. 3. Nucleotide sequence of the 9λ5 LTR and comparison of this sequence with other VL30 LTR sequences (see text). Comparisons with 9λ5 are denoted as follows: –, same sequence; N, nucleotide substitution; and Δ, deletion. The positions of insertions in the BVL-1 LTR are denoted by vertical lines above the nucleotide 5′ to the insertion, and the sequences of the insertions are above the vertical lines. The regulatory signals for transcription are labeled and boxed. The two sets of direct tandem repeats are underlined in bold arrows. Because 9λ5 is a cDNA from the 3′ end of a VL30 transcript, it contains only U3 and R sequences and the poly(A) site from the 3′ VL30 LTR.

derived from genomic clones. As the cDNA clone 9λ5 represents a transcriptionally active VL30 element, we determined the sequence of the 700-base-pair (bp) *Bgl*II-*Eco*RI fragment of 9λ5 (Fig. 2). We chose to sequence this part of the cDNA clone because alignment of pK5 and 9λ5 with the known restriction map of BVL-1 indicated that this 3′ portion contained the 3′ LTR.

The sequence of the 9λ5 LTR and a comparison of this sequence to some other VL30 LTR sequences are shown in Fig. 3. The 5′ end of the 9λ5 LTR is 192 bp downstream from the *Bgl*II site (Fig. 2). The 9λ5 LTR has both the U3 and R regions but, being a cDNA, does not contain the U5 region which is downstream of the poly(A) addition site. The sequences boxed in Fig. 3 enclose a TATA box, which is part of the polymerase II promoter, and the poly(A) addition sequence present in the VL30 LTRs. It has been shown that VL30 transcription initiates about 23 nucleotides downstream from this promoter sequence (31). Our studies show that for the 9λ5 transcript, poly(A) addition occurs 16 nucleotides downstream of the poly(A) addition sequence. Finally, the 9λ5 LTR has two different sets of direct tandem repeats (underlined in bold arrows in Fig. 3). The first set (129 to 233) contains three repeats, each 35 bp long. Two of these repeats are perfect and differ from the last by a single nucleotide change. The second set (385 to 467) contains two repeats, 42 and 50 bp long, which differ by two mismatched nucleotides. In addition, the 50-bp repeat contains two 3-bp and one 2-bp insertions.

Comparison of the 9λ5 LTR to the LTR of a genomic VL30 element called BVL-1 (15) shows good homology. The two major differences are that the BVL-1 LTR has a large insertion of 62 bp at position 76 and is missing an entire 35-bp repeat. Interestingly, these 35-bp repeats have homology to a pair of 72-bp repeats present in the Moloney murine sarcoma virus (15). In Moloney murine sarcoma virus, these 72-bp repeats function as enhancer sequences (27). The 9λ5 LTR has considerably less homology with the LTR of a VL30 element called NVL-3 (31). This finding was surprising, since both NVL-3 and 9λ5 represent VL30 genes that are transcriptionally active in NIH 3T3 cells. In fact, NVL-3 represents the most abundant type of VL30 RNA found to be copackaged into Moloney leukemia virus virion particles in NIH 3T3 cells (8). Interestingly, while the 5′ third of the U3 region and the whole R region are very homologous, it is the middle and 3′ third of the U3 region which are strikingly different between these LTRs. In 9λ5, this region contains the three 35-bp direct tandem repeats. These repeats are not present in NVL-3. It is not known if these 35-bp repeats are functioning as enhancers for 9λ5 transcription. If they are, this finding would suggest that in the case of NVL-3, other sequences, either in NVL-3 or in the flanking region, may be enhancing NVL-3 transcription.

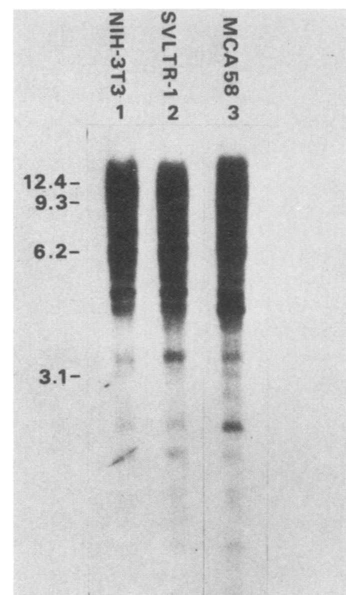

FIG. 5. Southern blot analysis of VL30 genomic elements homologous to 9λ5 in the NIH 3T3 and C3H cell lines. Each lane contains 10 μg of *Eco*RI-digested genomic DNA.

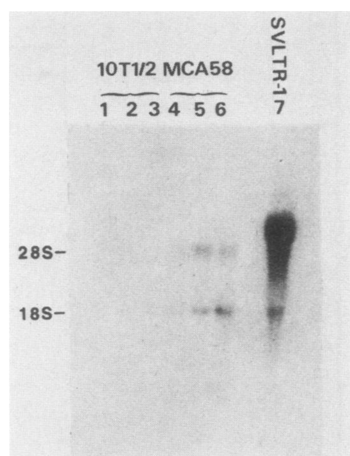

FIG. 4. Northern blot analysis of VL30 transcripts homologous to 9λ5 in the C3H cell lines. Each lane contains 20 μg of cytoplasmic RNA. The lanes are: (1) 10T1/2, nonconfluent; (2) 10T1/2, just confluent; (3) 10T1/2, confluent; (4) MCA 58, nonconfluent; (5) MCA 58, just confluent; (6) MCA 58, confluent; (7) SVLTR1, confluent. The positions of the 28S and 18S rRNAs are shown.

Expression of VL30 sequences in mouse C3H cells. We looked at the expression levels of VL30 RNAs in a C3H mouse cell line, 10T1/2 (33), and a methylcholanthrene-transformed derivative of it, MCA 58 (34). Using 9λ5 as a probe in Northern analysis, we were unable to detect any significant VL30 expression in these cell lines (Fig. 4). The faint bands detected in Fig. 4, lanes 4, 5, and 6, indicate cross-hybridization to 18S and 28S rRNAs. In a parallel lane, we show the level of VL30 transcripts found in the SV40-transformed NIH 3T3 cell line, SVLTR1. In another experiment, when we used the genomic clone pK5 as the probe, we obtained the same result. On very long exposures, we began to detect the VL30 30S transcript in the transformed cells (data not shown). It is not clear why VL30 expression differed so markedly between the C3H and NIH 3T3 cell lines, although there are a number of possible explanations which we will discuss later.

We also used 9λ5 as a probe of *Eco*RI-digested genomic DNA from these cell lines (Fig. 5). Interestingly, 9λ5 detected a number of bands, the majority of which were identical, in both the C3H and NIH 3T3 cell lines. The fact that there are no *Eco*RI sites in any of the VL30 elements sequenced to date suggests that the positions of the majority of VL30 genes are conserved in both these cell lines and that there is little polymorphism in the flanking DNA sequences. The chromosomal polymorphisms of the VL30 family with

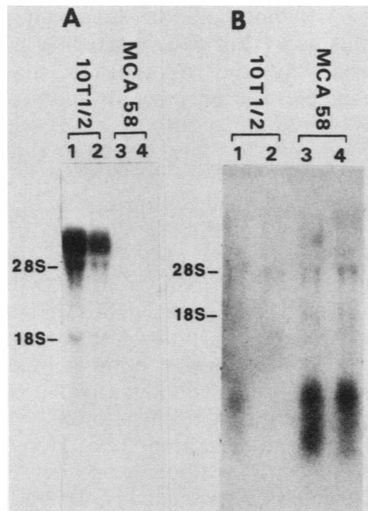

FIG. 6. Northern blot analysis of transcripts homologous to 4λ1 (A) and 10λ5 (B) in the C3H cell lines. Each lane contains 20 μg of cytoplasmic RNA. For each panel the lanes are: (1) 10T1/2, nonconfluent; (2) 10T1/2, confluent; (3) MCA 58, nonconfluent; and (4) MCA 58, confluent. The positions of the 28S and 18S rRNAs are shown.

respect to both position and internal heterogeneity warrant further study. It is clear that the blot shown in Fig. 5 indicates only that there are no major differences between the majority of those copies which are LTRs homologous to our cDNA.

Expression of 4λ1 and 10λ5 in mouse C3H cells. We studied the expression of two other of our cloned cDNAs in the C3H cell lines. The cDNA clone 4λ1 had a very different pattern of expression from that seen in the NIH 3T3 cell lines (Fig. 1G). In the NIH 3T3 lines, 4λ1 was well expressed only in confluent cells of both the transformed and parental lines. In contrast to our findings with the C3H cell lines, we found high levels of the large 4λ1 transcript in both the nonconfluent and confluent parental 10T1/2 cells (Fig. 6A, lanes 1 and 2). However, in the transformed MCA 58 cells, the levels of the 4λ1 transcript were low to undetectable (Fig. 6A, lanes 3 and 4; on long exposures, we began to detect the 4λ1 transcript in the confluent MCA 58 cells). In the C3H cell lines, 4λ1 therefore appears to represent a novel kind of gene whose expression is significantly reduced as a consequence of transformation.

We found the pattern of expression of the small B2 polymerase III transcripts homologous to 10λ5 to be similar in the C3H and NIH 3T3 cells; namely, the levels of these transcripts were elevated in the transformed cells (Fig. 6B, lanes 3 and 4 compared with lanes 1 and 2).

DISCUSSION

By constructing and screening a cDNA library made from SV40-transformed mouse cells, we isolated cDNAs representing genes that are differentially expressed between this transformed cell and the nontransformed NIH 3T3 parental cell. We found that only a small number of genes were differentially expressed. This finding is in agreement with earlier studies which compared the cytoplasmic mRNA populations of various normal cells with their transformed derivatives by mRNA-cDNA hybridization analysis (14, 47). These studies found that the majority of mRNAs are common to both the normal cell and its transformed counterpart. Two other groups have carried out experiments similar to ours. The results of Scott et al. (38) agree well with ours; however, surprisingly, Schutzbank et al. (37) found a much higher number of induced clones. These workers reported that more than 1 in 50 cDNA clones analyzed showed clear differences in expression between the SV40-transformed line and the parent. We do not understand these differences, but perhaps they relate to the facts that a specific fraction of mRNA was used in the study by Schutzbank et al. and that this fraction was purified for such genes.

The cDNAs we isolated exhibited various distinct types of differential expression (Fig. 1). The expression of most of these cDNAs appeared to be transformation specific or growth specific or both, although cDNAs with other, not so easily classifiable types of expression have also been isolated. These various patterns of expression could give rise to misleading results in studies aimed at the identification of genes differentially expressed because of viral transformation. This point can be demonstrated in the cases of the cDNA clones 4λ1 and 9λ5. If RNA from only one set of growth conditions was used to compare the parent and transformed cell, the clone 4λ1 could be classified as either a transformation-specific clone (Fig. 1G, compare lanes 2 and 3), an uninduced clone (Fig. 1G, lanes 2 and 4), or a clone expressed in nongrowing cells (Fig. 1G, lanes 1 and 4), depending on the density of the cells. Likewise, the growth-specific expression of the clone 9λ5 may have been classified as uninduced (Fig. 1E, lanes 1 and 3) or transformation specific (Fig. 1E, lanes 2 and 4). These results highlight the importance of taking into consideration growth-specific (and other cell cycle) changes in such studies.

Furthermore, we emphasize that the particular expression patterns detected for a given gene may be the result of complex interactions between many different cellular components and the gene under study, and therefore results may vary, depending on the genotypes and histories of the cell line. This variation can be seen in the case of both the 4λ1 and VL30 transcripts. (A summary of the expression patterns for 4λ1 and 9λ5 is shown in Table 1.) For example, we found the levels and patterns of 4λ1 expression to vary strikingly in different mouse cell lines. In the 3T3 and SVLTR1 cells, we found high levels of these 4λ1 transcripts in cells that had been at confluency for 2 days (Fig. 1G, lanes 2 and 4). In nonconfluent cells, we found low, barely detectable levels of these transcripts (Fig. 1G, lanes 1 and 3), and in cells that had just reached confluency, we found an intermediate level of 4λ1 expression (data not shown). However, in the C3H cell lines, we found a very different pattern of expression. We found high levels of the 4λ1 transcript in both the nonconfluent and confluent parental

TABLE 1. Expression of 9λ5 and 4λ1 in NIH 3T3 and C3H mouse cell lines

Clone	Transcript expression in[a]:							
	NIH 3T3		SVLTR1		$10T^{1/2}$		MCA 58	
	NC	C	NC	C	NC	C	NC	C
9λ5 (VL30)	+	−	+	+	−	−	−	−
4λ1	−	+	−	+	+	+	−	−

[a] +, High levels of transcripts; −, low to undetectable levels of transcripts; NC, nonconfluent; C, confluent.

10T1/2 cells, but low to undetectable levels in the transformed MCA 58 cells (Fig. 6A). It is clear that if the expression of 4λ1 in either the NIH 3T3 or C3H cell lines is considered independently of expression in the other, very different conclusions would be reached about the possible function of 4λ1 in the cell. While it is possible to speculate that the particular phenotypes of expression for 4λ1 play some role in the physiology of the cell, it is just as likely that the changes are epiphenomenal to growth regulation.

We isolated two related cDNA clones, 9λ5 and 14λ5, representing genes whose expression is growth specific in NIH 3T3 cells. Both these cDNAs were found to be derived from mouse VL30 transcripts. Moreover, in an earlier, somewhat different attempt to screen for differentially expressed genes, we also isolated a VL30 clone (see Results). We conclude that in mouse NIH 3T3 cells, a major class of differentially expressed genes is of those genes of the VL30 family. Our results are consistent with earlier studies which found an association between VL30 RNA levels and the proliferative state of the cell (2, 16, 39). More recently, an attempt was made to isolate mouse cellular genes whose expression was induced as a result of epidermal growth factor stimulation of quiescent AKR-2B mouse embryo cells (13). In this study, cDNA probes representing the polysomal-associated poly(A^+) RNA from either quiescent AKR-2B cells or AKR-2B cells 6 h after epidermal growth factor stimulation were used to screen an AKR-2B genomic library. Out of about 10^5 clones screened, only 3 consistently proved to be positive for differential expression. Strikingly, all three clones were found to contain VL30 sequences. It is now known that it is the VL30 30S transcript which is specifically induced by epidermal growth factor in these cells (10).

It is not known how many VL30 genes are sensitive to growth conditions or indeed how many are transcribed. Studies of the expression of the VL30 genes are complicated by the fact that there are about 100 to 200 genomic copies in these cells. On the basis of both the methylation patterns of VL30 genomic DNA (19) and a characterization of VL30 RNAs isolated from virion particles (8), it is thought that only a few members of this family are actively transcribed. However, it is not clear if the small number of types of VL30 elements found in virion particles reflects the number of VL30 genes transcribed or some defectiveness in *cis*-acting packaging signals.

In this context it is interesting that we found significantly lower levels of VL30 expression in the C3H cell lines than in the NIH 3T3 cell lines. Using the cDNA clone 9λ5 as a probe, we could not detect VL30 expression in either the chemically transformed MCA 58 cells or the parental 10T1/2 cells. This result was surprising, since it has been reported that VL30 exhibits transformation-specific expression in these cells (10). However, this earlier study did find the expression of VL30 in the transformed cells to be low. With the genomic clone pK5 as the probe, we detected VL30 expression in the transformed MCA 58 cells with long exposures. We empasize, however, that this expression was very low compared with that seen in the NIH 3T3 cell lines.

The molecular basis for this difference in VL30 expression between C3H cells and NIH 3T3 cells is unclear. Southern blot analyses of genomic DNA from both the NIH 3T3 and the C3H cell lines showed that both cell lines have approximately the same number of VL30 genes. Furthermore, the positions of the majority of these genes are conserved in both these cell lines. Perhaps only one or a few VL30 genes are capable of being highly expressed in response to growth conditions. This situation could be due to unique *cis*-acting sequences in this gene(s) or to its particular position in the genome. Alternatively, the genotypes of these cells may differ in, for example, the presence or activity of a cellular factor(s) involved in the response of VL30 gene expression to growth conditions. We are attempting to distinguish between these possibilities. Our preliminary studies suggest that the promoter contained within U3 in the 9λ5 LTR is functional (S. Saragosti, unpublished observations).

It is curious that two of the cDNAs clones that we found to show significant transformation- or growth-specific expression are derived from repeated gene families, namely the B2 and VL30 families. In a type of study similar to ours, Yamamoto et al. (49) isolated a number of cDNA clones representing genes expressed abundantly in a variety of lines of chemically induced rat ascites hepatomas but appearing at a minimal level, if at all, in normal liver. Here again a large number of the clones (44%) appeared to be derived from a class of middle repetitive sequences. In addition, mouse intracisternal A-particle genes, another repeated retrovirus-like gene family, were found to be abundantly expressed in many mouse tumor cells (11) and in early stages of mouse embryogenesis (7, 9), but rarely in normal mouse cells (48). Very recently it was also reported that in Friend erythroleukemia cells the expression of intacisternal A-particle LTR sequences are cell cycle linked (1). Finally, we found that the expression of another rodent repeated-gene family, the B1 family, is also somewhat sensitive to transformation (K. Singh, M. Carey, and M. Botchan, unpublished observations). Thus the cytoplasmic levels of transcripts from a number of rodent repeated families appear to be sensitive to growth conditions and the transformed state, in contrast to the majority of cytoplasmic transcripts. It is not clear why this situation exists, although it may be that the transposition of these elements is favored in actively growing cells. From an evolutionary point of view, this situation would seem to be advantageous only in the germ line. What is clear is that activation of a set of repeated and dispersed genes by transformation may give rise to secondary effects which contribute to the phenotype of some transformed cell lines or tumors (see, for example, references 25 and 32).

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