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Expression of Growth-Regulated Genes in Normal and SV40 Transformed Hamster Fibroblasts

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Abstract Transformation by the oncogenic virus SV40 has been shown to alter the expression of cellular genes at the level of RNA abundance. Many of these genes have yet to be identified. We have determined, by Northern blot analysis, the abundance levels of several growth-regulated genes in SV40-transformed cell lines to determine if their expression is altered and correlates with the ability of SV40 transformed cells to grow in low serum containing media. The mRNA abundance levels of the G_1-specific genes 2A9/calcyclin, 2F1/translocase, and 4F1/vimentin were determined in the parental hamster fibroblast cell line, tk⁻ts13, and in two SV40 transformants, HR5 and HR8 cells, grown in medium containing 10% calf serum (normal medium) and in HR5 and HR8 cells adapted to passage in medium containing low serum. A spontaneous transformant of the parental line capable of growth in low serum in the absence of SV40 transformation (tk⁻ts13/1%), was also included in these studies. The low serum adapted SV40-transformed cells and the spontaneous tk⁻ts13 transformed cells grew more vigorously than their nonadapted counterparts in medium containing low serum. The low serum adapted cells also grew to higher saturation densities in low serum and to densities comparable to those in high serum, whereas the nonadapted cells grew to low saturation densities in low serum, but not as low as the untransformed parental. These growth-regulated genes were expressed at lower levels in the SV40 transformed cells growing in medium containing high or low serum, and in the adapted parental cells (tk⁻ts13/1%) grown in medium containing low serum, in comparison with their levels in the nontransformed parental cells (tk⁻ts13/10%) grown in medium containing high serum. Therefore, the decreased levels in the expression of these growth-regulated genes could not be correlated to the rapid growth of SV40 transformed cells. We conclude that the molecular mechanism(s) that permits low serum adapted growth and SV40 transformed growth is different, at least in part, from the mechanism operating in nontransformed cells.

Key words: cell growth, gene expression, vimentin, calcyclin, ADP/ATP translocase, histone

Growth-regulated genes originally derived from Syrian hamster fibroblasts by differential screening of a cDNA library have been previously described (Hirschhorn et al., 1984a). These genes have been extensively studied in several cell systems and are referred to as G_1-specific genes because their cognate transcripts transiently increase in abundance in the G_1 phase of the cell cycle following serum stimulation from a quiescent state (Gibson et al., 1986). It has been suggested that the product of these genes (and others not yet identified) may be components of the mitogenic signal transduction pathway (Rollins and Stiles, 1989) and may contribute to tumor growth when their expression is somehow altered (Denhardt et al., 1986). The cellular homologs of a number of retroviral transforming oncogenes are induced when quiescent cells are mitogenically stimulated (Rollins and Stiles, 1989). The altered expression of these oncogenes has been shown to correlate with the transformed phenotype in some situations. Transformation by the oncogenic virus SV40 has been shown by a number of investigators to affect the expression of some cellular genes at the level of RNA abundance (Williams et al., 1977; Schutzbank et al., 1982; La Bella et al., 1983; Scott et al., 1983; Singh et al., 1985). Additionally, SV40 viral DNA has been shown to induce cellular DNA synthesis in G_1-specific temperature sensitive mutants of the cell cycle incubated at the restrictive temperature (Floros et al., 1981). Interestingly, it has been demonstrated that the expression of the cellular homologs of a number of oncogenes (*myc, mos,*

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erbB, erbA, ras, src, fps, and *abl*) was not altered in SV40 transformed Fischer rat fibroblasts. The expression of some cellular genes, identified by the differential screening of cDNA libraries constructed from mRNA isolated from SV40 transformed cells has been shown to be preferentially expressed in the transformed cells (Schutzbank et al., 1982; Scott et al., 1983; Singh et al., 1985). Other cellular genes have been shown to be preferentially expressed in the nontransformed cell. Furthermore, the transcription factor, SP1, has been shown to be expressed at higher levels in SV40 infected monkey kidney fibroblasts, suggesting that genes, whose expression may be regulated in part by SP1, may be more abundant in these cells, and may contribute to the transformed phenotype in SV40-transformed cells (Saffer et al., 1990). Transformation by SV40 may alter the expression of additional genes, like growth-regulated genes, that may be involved in signal transduction and are induced by the mitogenic stimulation of serum.

Previously, we constructed two SV40 transformed hamster fibroblasts (HR5 and HR8) and characterized the extent of their transformation by several criteria, including saturation density, ability to grow in soft agar, resistance to growth inhibition by butyrate or by dibutyryl-cAMP, and plating efficiencies (Hirschhorn et al., 1984b). The HR5 cells contained about twice the amount of SV40-specific RNA and large T antigen (required for transformation) when compared with the HR8 cells, even though they both contained about the same amount of SV40 specific DNA integrated into their genomes. By all criteria tested, HR5 and HR8 cells were more transformed than the parental cell line, tk$^-$ts13, and were comparable to each other in their growth characteristics. All of these studies had been done using cells continuously grown in optimal growth conditions, meaning in medium containing high serum. In the current study, we have determined the mRNA abundance levels of several growth-regulated genes in these SV40 transformed hamster fibroblasts grown in 10% serum (HR5/10% and HR8/10%) and in cells adapted to continuous growth in low serum (HR5/1% and HR8/1%) to determine if alterations in the expression of these genes can be correlated to SV40 transformation and to their ability to grow in low serum, when compared with the parental, nontransformed cells. A spontaneous transformant (tk$^-$ts13/1%) of the parental cell line (tk$^-$ts13/10%) was also included in this study.

METHODS

Cell Culture

The tk$^-$ts13 cell line, originally derived from the Syrian hamster cell line ts13 (Jonak and Baserga, 1980) was transfected with pC2, a plasmid containing the herpes simplex virus thymidine kinase and a complete SV40 genome (Shen et al., 1982). Several clones were selected in HAT media and those clones designated HR5 and HR8 were further characterized (Hirschhorn et al., 1984b). In this study, HR5/10% and HR8/10% indicates continuous growth in Dulbecco's modified Eagle's medium (MEM) containing 10% calf serum. These cells were then adapted to continuous growth in low serum by multiple passages, first in medium containing 2% fetal calf serum and then in medium containing 1% fetal calf serum. Medium containing low concentrations of calf serum were insufficient for continual growth of unadapted cells. HR5/2% and HR8/2% refer to cells maintained for many passages in medium containing 2% fetal calf serum, and HR5/1% and HR8/1% refer to cells maintained for many passages in medium containing 1% fetal calf serum. Control cultures of the parental line maintained in 2% fetal calf serum yielded a rare spontaneous transformant referred to as tk$^-$ts13/2%, which was further adapted to growth in medium containing 1% fetal calf serum. For routine growth cells were plated at 5×10^5 cells in a 100 mm plate in Dulbecco's (MEM) supplemented with serum as indicated for each experiment, and incubated at 34°C in a 5% CO_2 humidified atmosphere. For growth studies, cells were plated at 3×10^5 per 60 mm dish in Dulbecco's (MEM) containing serum as indicated. At the indicated times, duplicate plates were harvested and the cell density determined by counting. Saturation densities were determined after 4 days growth.

RNA Isolation and Blot Hybridizations

Total cytoplasmic RNA was isolated from exponentially growing cells following lysis with 150 mM NaCl, 10 mM Tris-HCl, pH 7.9, at 4°C, 1.5 mM $MgCl_2$, 0.65% Nonidet-P40, and 10 mM ribonucleoside-vanadyl complex, essentially as previously described (Hirschhorn et al., 1984b). Following purification by phenol/chloroform extraction and ethanol precipitation, the RNA was

size-fractionated by 1% agarose/2.2 M formaldehyde gel electrophoresis, transferred to nitrocellulose, and hybridized overnight at 42°C to nick-translated probes as indicated (Maniatis et al., 1988). Equivalent amounts of RNA were loaded as determined by spectrophotometric analysis and verified by UV shadowing as described (Steiner et al., 1991). Blots were washed twice at room temperature in 2 × SSC/0.1% SDS, followed by two washes at 52°C in 0.1 × SSC/0.1% SDS. Blots were exposed to X-ray film at −80°C in the presence of an intensifying screen. Signal intensities were assessed by densitometric scanning of each signal, which was then adjusted to the signal obtained using the histone H3 G_1/S specific probe, FO422, to account for differences in the cycling populations (Calabretta et al., 1986b). The following probes, 2A9, 2F1, 4F1, and 11D6, were originally obtained from a Syrian hamster cDNA library and have been described (Hirschhorn et al., 1984a; Gibson et al., 1986); FO422 is a genomic clone of histone H3 kindly supplied by Dr. Gary Stein; pSV2G (Galanti et al., 1981) contains the entire SV40 genome cloned into the Bam HI site of pBR322 and was obtained from Dr. Renato Baserga.

RESULTS

One of the striking characteristics of transformed cells in culture is their ability to grow in the absence of a large concentration of growth factor, usually supplied as a serum supplement. Non-transformed cells normally will enter a non-growing phase, G_0, and remain quiescent when incubated in medium containing low serum or medium deficient in growth factors (Baserga, 1985). Increased levels of growth factors induce these non-transformed cells to reenter the cell cycle, an event which requires the increased synthesis of specific genes (reviewed in Rollins and Stiles, 1989). The transformation event, by mechanisms only beginning to be understood, allows the cells to by-pass the low serum block. It is possible that genes involved in the transmission of the mitogenic signal in non-transformed cells may also be involved in the pathway which allows transformed cells to grow in low serum. We have tested this hypothesis by comparing the mRNA abundance levels of several growth-regulated genes in the SV40 transformed cells, HR5 and HR8, and cell lines adapted to continuous growth in medium containing low serum, with the levels in the nontransformed parental cells, tk^-ts13/10% cells.

The growth-regulated genes selected for this study, summarized in Table I, includes genes preferentially expressed in the G_1-phase of the cell cycle, as well as one expressed at the G_1/S border, and a nongrowth-regulated gene. The human cDNA homologues of the hamster growth regulated sequences p2A9, p2F1, p4F1 have been isolated and identified. p4F1 has been identified as the cytoskeletal intermediate filament, vimentin (Ferrari et al., 1986). p2A9, currently known as calcyclin, is a member of the S100 family of calcium binding proteins (Calabretta et al., 1986a; Ferrari et al., 1987; Filipek et al., 1990) and was shown to be identical to the prolactin receptor associated (PRA) protein (Murphy et al., 1988). p2F1 has been shown to be an ADP/ATP translocase (Battini et al., 1987). These G_1-specific genes have been shown to be transiently induced by the mitogenic stimulation of fibroblasts and lymphocytes (Hirschhorn et al., 1984a; Gibson et al., 1986). These genes have also been shown to be differentially expressed in human leukemias, further implicating their involvement in aberrant cell growth (Calabretta et al., 1985; Calabretta et al., 1986b). Other genes used in this study include FO422, a genomic clone of histone H3, a G1/S phase specific gene sequence (Hirschhorn et al., 1984c), p11D6, which is an unidentified nongrowth-regulated gene sequence (Gibson et al., 1986), and pSV2G, which contains the entire early region of SV40 DNA in pBR322 (Galanti et al., 1981).

Growth Characteristics of Adapted Cell Lines

Previously, we constructed two transformed cell lines, HR5 and HR8, by transfecting a hamster fibroblast cell line with a molecularly cloned SV40 genome (Hirschhorn et al., 1984b). The growth characteristics of these cells strongly

TABLE I. Growth-Regulated Genes Used in This Study

Plasmid	Identified protein	Time of maximum expression	Size of mRNA (nt)
2A9	calcyclin	G_1	900
2F1	translocase	G_1	1,500
4F1	vimentin	G_1	1,800
FO422	histone H3	G_1/S	500
11D6	unknown	nongrowth-regulated	2,800
SV2G	SV40	constitutive	4,000

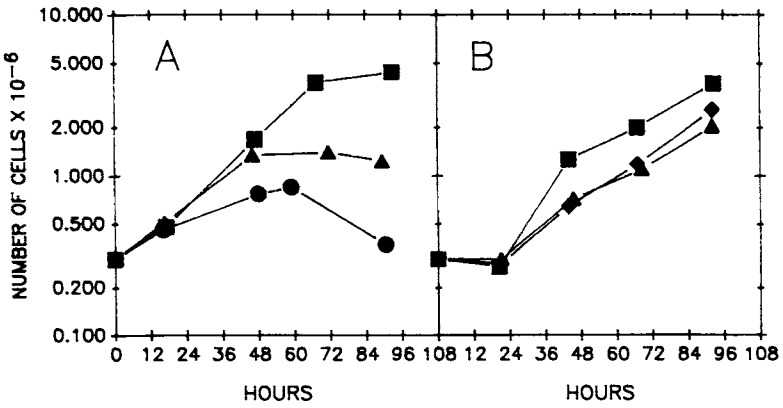

Fig. 1. Growth curves of the HR5 Cell Lines. **Panel A:** Cells continuously passaged in medium containing high (10%) calf serum were plated at 3×10^5 per 60 mm dish in growth media containing 0.1% (circles), 1% (triangles), or 10% (squares) calf serum in Dulbecco's modified Eagle's medium. At the indicated times, duplicate plates were harvested as described in Materials and Methods, and the cell density was determined. **Panel B:** Cells adapted to growth in medium containing 2% fetal calf serum, and continuously passaged in medium containing 2% fetal calf serum, were plated as above in medium containing 1% (triangles), 2% (diamonds), or 10% (squares) calf serum in Dulbeccos' modified Eagles' medium. Cells were harvested as above. These graphs are representative of duplicate experiments.

suggested that these cells are fully transformed. However, when these cells are continuously passaged in medium containing 10% calf serum and then are transferred to medium containing 1% calf serum, they do not attach well to the plates and do not grow vigorously (Figs. 1A, 2A). It was previously shown that, although HR5 and HR8 are clonal populations, the SV40 T antigen (which is required for transformation) was heterogeneously expressed (Hirschhorn et al., 1984b), which could account for their limited growth ability when immediately challenged with low serum. To be able to analyze the expression of growth-regulated genes in cells grown under different conditions, it was first necessary to adapt the HR5 and HR8 cells to continuous growth in medium containing low serum. The HR5 and HR8 cells were first plated in medium containing 10% calf serum. After these cells attached, the cells were rinsed and medium containing 2% fetal calf serum was added. After 6 passages in medium containing 2% fetal calf serum, the cells grew vigorously. These adapted cells (HR5/2% and HR8/2%) grew equally well in medium containing low or high serum (Figs. 1B, 2B). The parental, nontransformed tk^-ts13 cells (tk^-ts13/10%) were treated similarly. Although most of these cells died, as was expected, since non-transformed cells require medium with high concentrations of growth factors (Baserga, 1985), a single colony grew out that we continued to carry and adapt to lower concentrations of serum (tk^-ts13/2%). This process appears to be similar to the progressive state selection of NIH 3T3 cells described previously (Rubin et al., 1990), where cells acquire an increased capacity

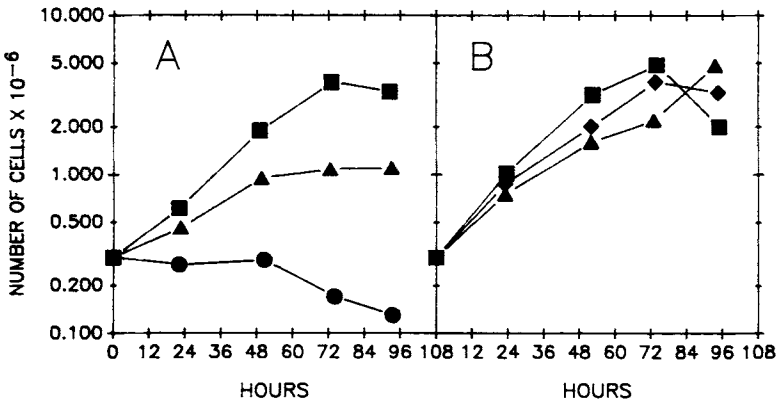

Fig. 2. Growth curves of the HR8 cell lines. **Panel A** and **Panel B** are as described in Figure 1.

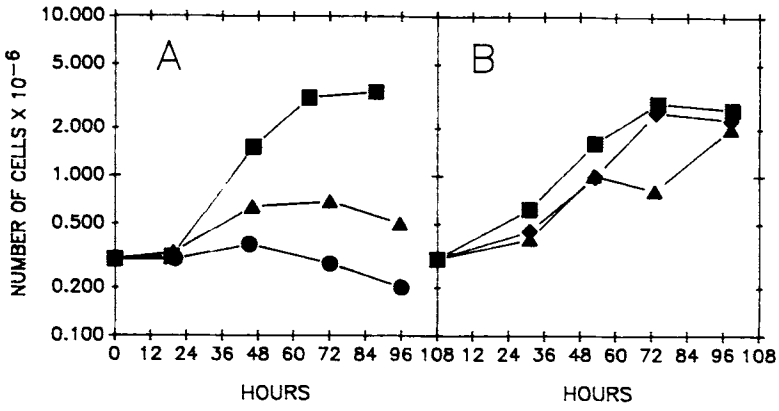

Fig. 3. Growth curves of the tk⁻ts13 cell lines. **Panel A** and **Panel B** are as described in Figure 1.

for proliferation when maintained in growth constraining conditions. These adapted tk⁻ts13 cells grew equally well in medium containing low and high serum concentration (Fig. 3B), whereas the nonadapted parental cells (tk⁻ts13/10%) only grew in medium containing high serum (Fig. 3A). While all the nonadapted cell lines grew equally well in the medium containing 10% calf serum, the nonadapted SV40 transformed cells (HR5/10% and HR8/10%) could grow in medium containing 1% calf serum, but at only half the maximal rate.

The growth characteristics of these cells are summarized in Table II. Continuous growth in medium containing low serum allowed the adapted cells to achieve a higher saturation density when grown in medium containing low serum than when the nonadapted cells were grown in low serum. All three nonadapted lines had longer doubling times when transferred directly to the lower serum concentrations in comparison with the doubling times of the adapted cells continuously growing in medium containing low serum. The growth characteristics of the adapted nontransformed parental cell line (tk⁻ts13/2%) were comparable to those of the adapted SV40 transformed cell lines (HR5/2% and HR8/2%).

TABLE II. Growth Characteristics of Cell Lines

	Doubling Times (hrs)		Saturation Density ($\times 10^6$)	
Cell lines	In 10%	1%	In 10%	1%
HR5 10%	16.9	22.3	4.4	1.4
HR5 2%	10.1	20.7	3.7	2.0
tk⁻ts13 10%	11.2	50.2	3.4	.7
tk⁻ts13 2%	17.0	16.8	2.9	2.0
HR8 10%	17.3	26.2	3.8	1.1
HR8 2%	18.7	20.2	4.8	4.8

Expression of Growth-Regulated Genes

The adapted HR5 and HR8 cells (HR5/2% and HR8/2%) and the spontaneously transformed tk⁻ts13 (tk⁻ts13/2%) were further adapted to grow in medium containing 1% fetal calf serum, using a similar protocol, prior to the isolation of RNA. Total cytoplasmic RNA was isolated from subconfluent cell monolayers in order to avoid potential differences resulting from excessive cell contact and to harvest cells when they were still vigorously growing. The RNA populations were fractionated on a 1% agarose-formaldehyde gels and blotted to nitrocellulose as described in Materials and Methods. The abundance levels of the growth-regulated genes was then determined by successive hybridization (Fig. 4). Except for the lack of SV40 specific RNA in both the tk⁻ts13 cell lines, all of the probes used were able to hybridize to all the RNA populations. 2A9/calcyclin, which is a Ca^{++} binding protein (Filipek et al., 1990) and has been demonstrated to be deregulated in acute myeloid leukemias (Calabretta et al., 1985, 1986b) showed one band of slightly less than 1 kb. 2F1/translocase, the hamster homolog of the mitochondrial ADP/ATP translocase (Battini et al., 1987), showed one band of approximately 1.5 kb. 4F1/vimentin detected one band of approximately 1.8 kb. FO422, which is a cloned human H3 histone gene and known to be expressed at G_1/S, was used as a marker to assess the extent of cell cycling within the population (Calabretta et al., 1986b). 11D6, a non-growth-regulated sequence (Gibson et al., 1986), was used to determine the effect, if any, on a

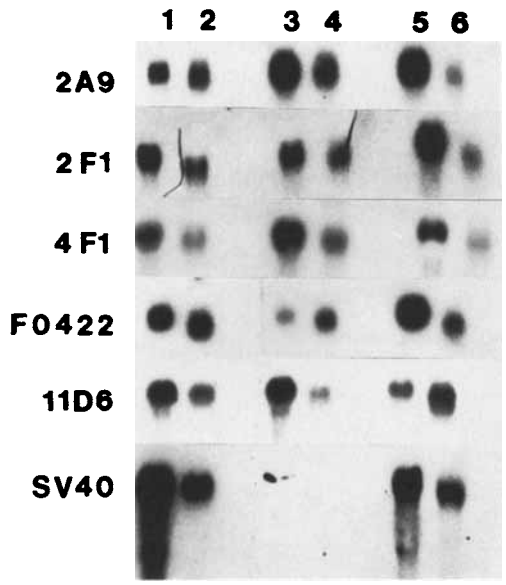

Fig. 4. Abundance levels of growth-regulated genes. The three nonadapted cell lines (HR5/10%, tk⁻ts13/10%, and HR8/10%) and the three adapted cell lines (HR5/1%, tk⁻ts13/1%, and HR8/1%) were each grown in large quantities in their respective media. Total cytoplasmic RNA was isolated from subconfluent cell monolayers. Total cytoplasmic RNA (~10 μg per lane) isolated from exponentially growing cells: **Lane 1,** HR5/10%; **lane 2,** HR5/1%; **lane 3,** tk⁻ts13/10%; **lane 4,** tk⁻ts13/1%; **lane 5,** HR8/10%; and **lane 6,** HR8/1%. The RNA was fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized to nick translated cDNA probes and washed as described in Materials and Methods. Blots were exposed to x-ray film at −80°C in the presence of an intensifying screen. This composite northern is representative of duplicate experiments.

gene not implicated in growth regulation. pSV2G, which contains the SV40 genome, was detected predominantly as a 4 kb band in the two SV40 transformed lines and their adapted counterparts. Since the tk⁻ts13 lines are not SV40 transformed, no transcripts were detected. The lack of SV40 specific transcripts in the adapted tk⁻ts13 verifies that these cells did not gain a growth advantage by inadvertent contamination with the SV40 transformed cells.

The signal intensities of the growth-regulated genes were quantitated by scanning laser densitometry and then normalized to the G_1/S phase specific gene, histone H3 (FO422) to account for differences in the growth fraction of the different cell populations. This was done to assure that any differences in gene expression would result from an alteration in the regulation of the gene expressed in these cells grown in the different conditions and not from differences in the cycling populations (Calabretta et al., 1986b). When examined in this way (Fig. 5), several observations can be made. The relative abundance levels of the growth-regulated genes in the SV40 transformed fibroblasts, regardless of the growth conditions, are lower than those in the parental tk⁻ts13 cells (tk⁻ts13/10%) grown in medium containing high serum. Adaptation of the tk⁻ts13 to growth in low serum (tk⁻ts13/1%) reduced the relative abundance levels of these growth-regulated genes to levels very similar to those seen in the SV40 transformants. The differences in the relative abundance levels of the growth-regulated genes are not striking, except for 2A9/calcyclin, which is almost tenfold higher in the nontransformed parental line (tk⁻ts13/10%) than in the SV40 transformants.

DISCUSSION

Transformation by the oncogenic virus SV40 has been shown to alter the expression of cellular genes at the level of RNA abundance (Williams et al., 1977). Approximately 3% of the transcripts present in the transformed cell were not present at detectable levels in the progenitor line, while about 5% of the transcripts were preferentially expressed in the nontransformed parental cell line. A few of the genes preferentially expressed in the transformed cells have been identified by the differential screening of cDNA libraries constructed from mRNA isolated from SV40 transformed cells (Schutzbank et al., 1982; La Bella et al., 1983; Scott et al., 1983; Singh et al., 1985). The identity of most of these genes has not been determined. It has also been demonstrated that the expression of several cellular oncogenes (including *myc, mos, erbB, erbA, ras, src, fps,* and *abl*) is not altered following SV40 transformation of rat fibroblasts (Winberry et al., 1985). The fact that the SV40 T antigen has been shown to bind to the retinoblastoma gene product and presumably inactivate its normal growth suppressing function (DeCaprio et al., 1988; Ludlow et al., 1989) suggests that the expression of growth-regulated genes might be affected in SV40 transformed cells, even in the absence of serum stimulation. The transcription factor SP1 has been shown to be transcribed at a higher level in SV40 infected monkey kidney fibroblasts, suggesting that the increased transcription of genes containing SP1 recognition sites in their promoters may also contribute to the transformed phenotype (Saffer et al., 1990). The promoters of the growth regulated genes tested in this study have all been

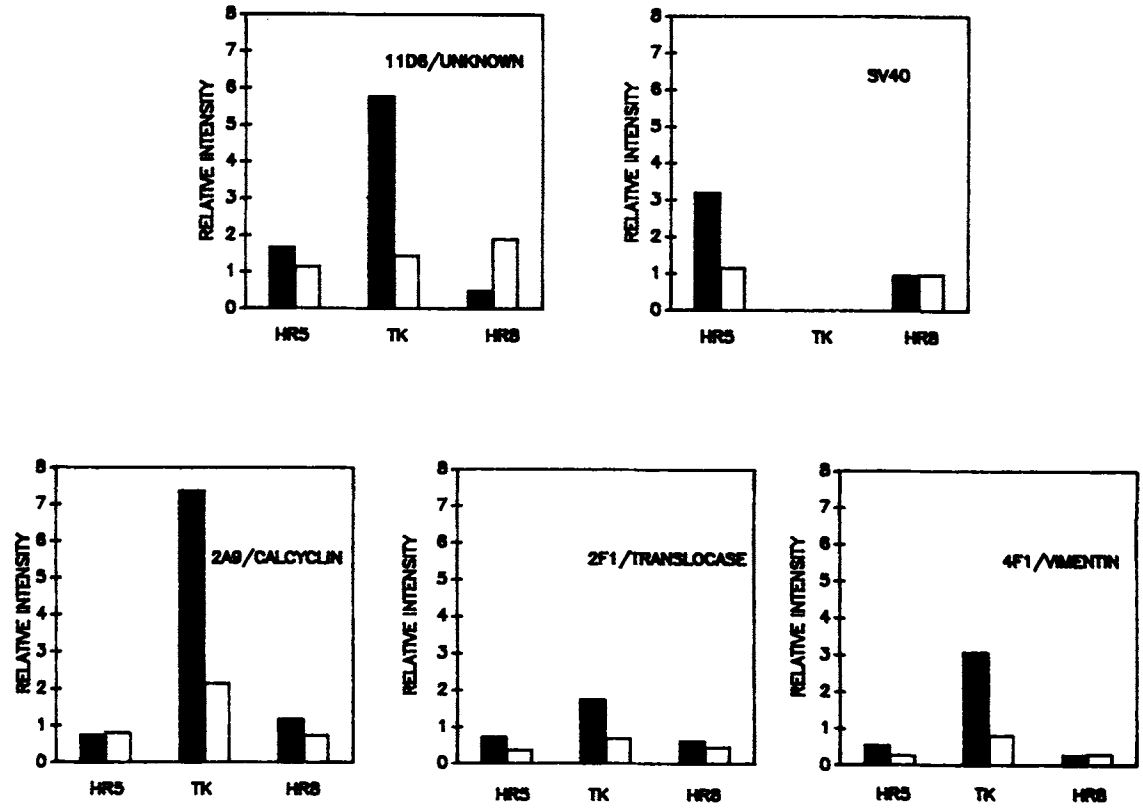

Fig. 5. Relative abundance levels of growth-regulated genes. The intensities of the bands in Figure 4 were quantitated by scanning laser densitometry of each band, as described in Materials and Methods. Closed bars represent samples from nonadapted cells grown in 10% serum and open bars represent samples from adapted cells grown in 1% serum.

shown to contain at least one SP1 recognition site, and they are therefore good candidates for an altered expression in SV40 transformed cells (Rittling and Baserga, 1987; Ferrari et al., 1987; Ku et al., 1990). It has been suggested that cellular DNA synthesis induced by SV40 DNA in G_1-specific temperature sensitive mutants of the cell cycle at the restrictive temperature may result from a mechanism that overlaps with, but is distinct from, that of normal cells induced by serum growth factors (Baserga and Ferrari, 1987). Although the mechanism(s) of normal cell growth is only beginning to be understood at the molecular level, it is commonly believed that a temporal order of gene expression, including a subset of growth-regulated genes, is involved (Denhardt et al., 1986; Rollins and Stiles, 1989). The growth-regulated genes used in this study (4F1/vimentin, 2F1/translocase, and 2A9/calcyclin) were originally identified by the differential screening of a cDNA library constructed from mRNA isolated from serum stimulated fibroblasts. These genes have been shown to be growth-regulated in many cell systems (Gibson et al., 1986) and have been suggested to play a role in growth regulation. It has been suggested that in addition to their role in growth regulation of cells in culture, the expression of these genes in vivo may be related to tissue-specific function rather than proliferation potential (Soprano et al., 1987). Furthermore, these genes have been shown to be differentially expressed in human leukemias (Calabretta et al., 1985, 1986b). These findings suggest that an altered expression of these growth-regulated genes might contribute to the transformed phenotype. In this study, the abundance levels of these growth regulated genes in SV40 transformed cells has been determined and compared with the abundance levels found in the parental cell line and in SV40 transformed cells adapted to growth in medium containing low serum.

Previously, we had constructed the SV40 cell lines, HR5 and HR8, by transfection of tk$^-$ts13 cells (Hirschhorn et al., 1984b). These SV40 transformants were shown by several criteria to

be fully transformed. Cells from each line were adapted to growth in low serum (Figs. 1, 2 and Table II). At the same time, the parental tk⁻ts13 cells (tk⁻ts13/10%) were challenged with medium containing low serum and a single transformed clone grew out. These cells were adapted to continuous growth in medium containing low serum (tk⁻ts13/2%) and exhibited growth characteristics quite similar to those of the low serum adapted HR5 and HR8 (Fig. 3 and Table II).

Northern analysis of RNA isolated from HR5, HR8, and tk⁻ts13 cells grown in medium containing high or low serum demonstrated several things. First, all the growth-regulated genes tested hybridized to transcripts in all the RNA samples. Second, when the signal intensities, as determined by scanning laser densitometry, were normalized to that of the histone signal intensity (to correct for differences in the growth fraction of the different cell populations), the relative levels of 2F1/translocase, 4F1/vimentin, and 2A9/calcyclin were all lower in the SV40 transformed cells than in the parental tk⁻ts13 cells (tk⁻ts13/10%). Third, the relative abundance levels of 2F1/translocase, 4F1/vimentin, and 2A9/calcyclin in the adapted tk⁻ts13 (tk⁻ts13/1%) were lower than in the parental cell line, and were similar to that in the SV40 transformed cells. Fourth, although the relative abundance levels of all the growth-regulated genes tested were lower in the SV40 transformed cells than in the parental tk⁻ts13, the only growth-regulated gene that showed a marked difference was 2A9/calcyclin. Since the function, activity, and cellular location of calcyclin are not yet known, the significance of this difference is unclear. It is possible that the lower levels of calcyclin in the SV40 transformed cells and the adapted tk⁻ts13 cells relates to a decreased need for calcium-binding capacity, but there is no direct evidence of this. The relative levels of 4F1/vimentin were also lower in the SV40 transformed cells and in the adapted tk⁻ts13, but not as markedly different as the 2A9/calcyclin. Since vimentin is a component of the cytoskeleton, a decrease in its expression suggests a change in the organization of the cytoskeleton. There is ample evidence of changes in the morphology and cytoskeleton of transformed cells (Bershadsky and Vasiliev, 1988).

Continuous growth of SV40 transformed Syrian hamster fibroblasts in medium containing low serum (HR5/1% and HR8/1%) did not result in the extinction of expression nor overexpression of the growth-regulated genes studied when compared to the transformed (HR5/10% and HR8/10%) or parental (tk⁻ts13) cells grown in medium containing 10% serum. Adaptation of a nontransformed Syrian hamster fibroblast (tk⁻ts13/10%) to growth in medium containing low serum (tk⁻ts13/1%) allowed these selected cells to bypass a low serum block in the absence of SV40 and resulted in the decreased expression of all the growth-regulated sequences. The expression of growth-regulated genes in the adapted tk⁻ts13 was similar to the levels in SV40 transformed cells. The ability of SV40 transformed Syrian hamster fibroblasts (HR5 and HR8) and of the adapted Syrian hamster fibroblast (tk⁻ts13/1%) to grow in medium containing low serum could not be correlated to the extinction of expression nor overexpression of these G_1-specific growth-regulated sequences. However, the expression of 4F1/vimentin, 2F1/translocase, and 2A9/calcyclin was lower in the SV40 transformed cell lines and the adapted parental cell line. These differences might simply be the cells response to reduced serum levels. Alternatively, the significant decrease in the expression of 2A9/calcyclin in the transformed cells might be necessary for low serum growth. These results suggest that adaptation of growth in reduced serum or from SV40 transformation is by a distinct mechanism from that induced by the addition of 10% serum to quiescent cells. Whether or not these mechanisms overlap at the molecular level remains to be determined.

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