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Tumorigenicity of Fibroblast Lines Expressing the Adenovirus E1a, Cellular p53, or Normal c-*myc* Genes

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Cellular and viral oncogenes have been linked to the transformation of established cell lines in vitro, to the induction of tumors in vivo, and to the partial transformation or immortalization of primary cells. Based on the ability to cooperate with mutated *ras* oncogenes in the transformation of primary cells, the adenovirus E1a and cellular p53 genes have been assigned an immortalizing activity. It is demonstrated in this paper that the adenovirus type 5 E1a gene and simian virus 40 promoter-linked p53 cDNA are able to transform previously immortalized cells to a tumorigenic phenotype without a significant change in cell morphology. It is also shown that, when linked to a constitutive promoter, the normal mouse and human c-*myc* genes have the same transforming activity. Cells transformed by each of these oncogenes have an increased capacity to grow in the absence of growth factors and a limited anchorage-independent growth capability.

Cellular and viral oncogenes have been classified as immortalizing or transforming genes based on their behavior in certain assays and on the morphology they impart to cells that harbor them in an activated or constitutively expressed state. Thus, the cellular *myc* gene, the adenovirus (Ad) early region 1A (E1a), the polyomavirus large-T-antigen gene, and the cellular p53 gene have been classified as having immortalizing activity based on their ability to cooperate with an activated *ras* gene in the transformation of primary cells (2, 7, 16, 19, 22) and on their ability to rescue primary cells from senescence without inducing tumorigenicity (10, 11, 18, 23). The *ras* oncogenes and the polyomavirus middle-T-antigen gene have been placed under the transforming category as a result of their ability to transform immortalized cells (16, 26, 27, 29), imparting a characteristically refractile and rounded morphology.

Based on recent findings, however, it is becoming apparent that this classification is all too simplified. It has recently been reported that either a mutated H-*ras* gene or a long terminal repeat (LTR)-driven normal H-*ras* gene was able to rescue primary cells from senescence without any morphological transformation (35). In a previous study from our laboratory (12), we reported that mouse plasmacytoma c-*myc* genes which had been linked to viral promoters and transfected into fibroblasts were capable of transforming recipient cells without significantly altering their morphology. These results suggest that many activated oncogenes have both immortalizing and transforming activities, depending on the assay. This prompted us to carry out similar studies with constitutively expressed normal *myc* genes and other genes with which immortalizing activity has been associated, namely the Ad E1a and cellular p53 genes.

In this study we report that the Ad5 E1a gene, a simian virus 40 early-region promoter-driven mouse p53 gene, classified as immortalizing genes under some assay conditions, as well as normal human and mouse c-*myc* genes driven by strong promoters, are capable of inducing in mouse NIH/3T3 fibroblasts many properties of transformed cells without a greatly altered morphology.

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MATERIALS AND METHODS

Constructs, transfection, and selection. The LTR-hmyc plasmid was constructed from a clone of the normal human c-*myc* gene (*Hin*dIII-*Eco*RI fragment) which was isolated from a human gene library (17) and subcloned into the corresponding sites in pBR322. The *Xba*I site in the first c-*myc* intron was converted to a *Hin*dIII site by using linkers, and the *Hin*dIII-*Xba*I fragment that contained the 5′ flanking sequences and the first exon was excised and replaced with a retroviral LTR derived from the pEVX vector (a 1.1-kilobase [kb] *Hin*dIII fragment containing the left LTR and retroviral splice donor sequence [15]). The SVmmyc clone was derived from the SVc-myc clone (16) by replacing the plasmacytoma-derived c-*myc* gene from SVc-myc (*Xba*I-*Bam*HI fragment) with the corresponding fragment from a normal mouse c-*myc* clone (kindly provided by Roger Perlmutter and Lee Hood). The Ad 5 E1a plasmid, p1A, was a gift from Jonathan Logan and Tom Shenk, and p11-4 was a gift from John Wallis and Arnold Levine.

Using a calcium phosphate transfection protocol (9), plasmid DNAs were cotransfected into NIH/3T3 cells on 60-mm dishes in a 10:1 ratio with pSV2neo (34). After 36 h, the cells were trypsinized and expanded into 100-mm dishes, and G418 was added to the media containing 10% donor calf serum 24 h later at a concentration of 400 μg/ml. G418-resistant colonies were scored and picked after 2 weeks.

Growth rates, soft-agar cloning, and tumorigenicity. The growth rates of the control and transfected cells lines in medium containing 0.5 and 10% calf serum were determined as described previously (12). Anchorage-independent growth efficiencies in 0.26% soft agar were determined as described previously (12). Tumorigenicity assays were based on the procedure of Blair et al. (5). Cell lines were suspended in sterile phosphate-buffered saline at a density of 10^7/ml after having been harvested by trypsinization and washed at least twice in sterile phosphate-buffered saline. Cells (2×10^6) were injected subcutaneously into each *Balb*/c *nu*/*nu* mouse, and animals were monitored at regular intervals for the appearance of tumors.

Southern and Northern hybridization. RNA and DNA from cultured cells and tumors were isolated as described previ-

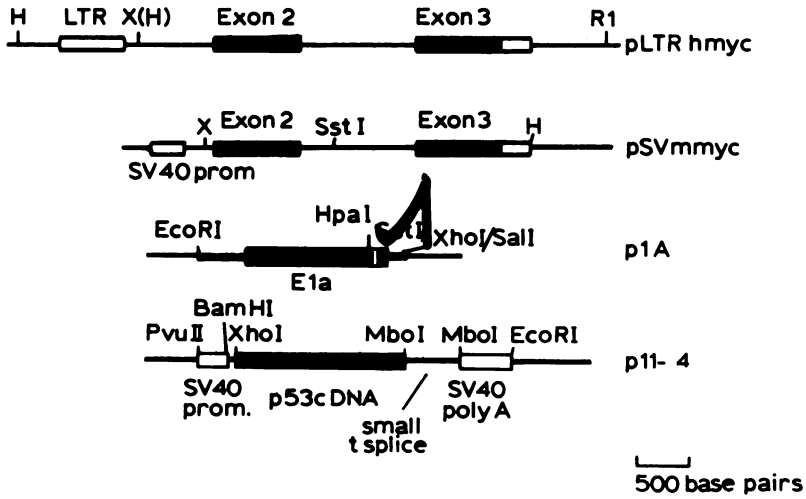

FIG. 1. Diagram of the constructs used in these studies. Plasmid pLTRhmyc contains the two human c-*myc* protein-coding exons, approximately 0.7 kb from the first intron and the polyadenylation signal. A murine leukemia virus LTR has replaced the first exon of the gene. In the pSVmmyc plasmid the two coding exons of a normal mouse *myc* gene are transcribed with the SV40 early-region promoter. The c-*myc* polyadenylation sites are retained. Plasmid p1A harbors the entire E1a region of Ad5 and about 60 base pairs from the E1b region. Plasmid p11-4 contains a p53 cDNA insert driven by an SV40 promoter (prom). A small t splice provided by the *Mbo*I insert and SV40 polyadenylation signals complete the plasmid. Restriction enzyme sites are indicated as: H, *Hin*dIII; R1, *Eco*RI; X, *Xba*I.

ously (13). For the Southern blot in Fig. 2A, *Eco*RI-digested DNAs were electrophoresed in 0.7% agarose, transferred to nitrocellulose by the method of Southern (33), and hybridized to a nick-translated 0.98-kb *Xba*I-*Sst*I restriction fragment serving as a second *myc* exon probe. For the blot in Fig. 2B, the probe used was a nick-translated 1.7-kb *Eco*RI-*Sst*I fragment isolated from the plasmid p1A. In Fig. 2C the probe used was a 1.1-kb *Stu*I fragment isolated from the p53 cDNA plasmid p11-4. All the hybridizations were carried out in the presence of 10% (wt/vol) dextran sulfate. After hybridization, filters were washed as described earlier (28). For Northern blots, poly(A)-containing RNAs were electropho-

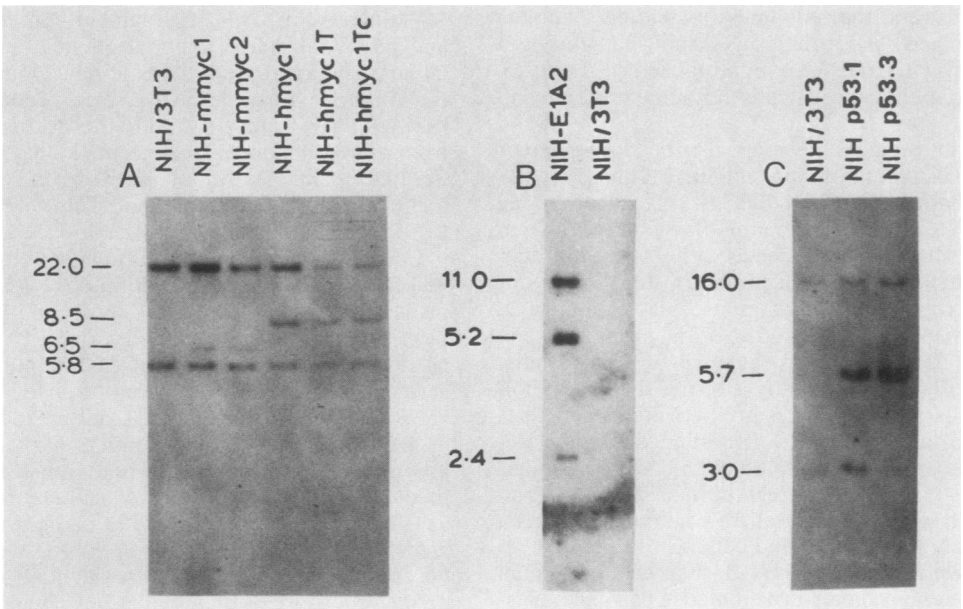

FIG. 2. Southern blot analysis from NIH/3T3 cells and transfected clones. (A) *Eco*RI digests of NIH/3T3, NIH m*myc*1, NIH m*myc*2, NIH h*myc*1, NIH h*myc*1T (tumor), and NIH h*myc*1Tc (cells reestablished in culture from a tumor) were electrophoresed and transferred to nitrocellulose. The blots were hybridized with a nick-translated 0.98-kb *Xba*I-*Sst*I fragment from a mouse c-*myc* gene. The 22.0-kb mouse endogenous band is indicated as well as the exogenous bands derived from transfection of either the pSVmmyc or the pLTRhmyc construct. (The 5.8-kb band common to both control and transfected cells is a c-*myc*-related sequence currently under study.) m*myc*1 and m*myc*2 are two different clonal lines, while h*myc*1T and h*myc*1Tc are both derived from the h*myc*1 clone. (B) *Eco*RI digests of NIH/3T3 and NIH E1a2 hybridized to a 1.7-kb *Eco*RI-*Sst*I fragment specific for E1a. (C) *Eco*RI digests of NIH/3T3, NIH p53.1, and NIH p53.3 hybridized to a 1.1-kb *Stu*I fragment from p11-4. The 16.0-and 3.0-kb endogenous bands as well as the exogenous bands from the transfected constructs are indicated. The 3.0-kb band is a p53 pseudogene (42).

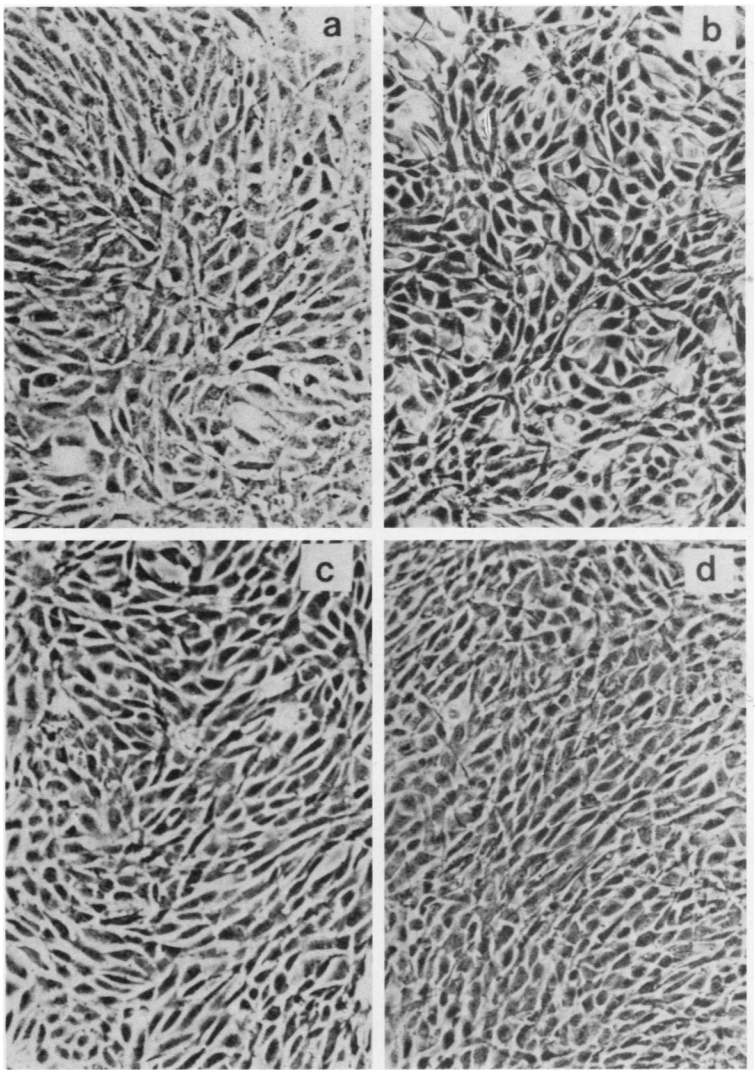

FIG. 3. Morphology of transfected NIH/3T3 fibroblasts. The cells were all photographed (magnification, ×200) at confluence in Dulbecco modified Eagle medium containing 10% fetal calf serum. (a) NIH neo1; (b) NIH h*myc*1; (c) NIH E1a2; and (d) NIH p53.3.

resed in 0.7% formaldehyde–agarose gel (24) and processed as previously described (28). Hybridization probes for Fig. 5A and B and 6 included the three described above, respectively, and for the Northern blot in Fig. 7 a *Bam*HI-*Bgl*II fragment specific for the first *myc* noncoding exon (4) was used.

RESULTS

Construction of plasmids and selection of cell lines. Previous studies from this laboratory investigated the transforming activity of c-*myc* oncogenes that had been derived from translocated alleles in mouse plasmacytomas (12). In the present study, two other oncogenes were tested for transforming activity as well as activated normal c-*myc* genes from both mice and humans. The Ad5 E1a gene was carried on a pBR322-derived plasmid, p1A, containing 1 to 15 map units of the Ad 5 genome from which all but 20 amino acids of the E1b region have been deleted (constructed by J. Logan). The second oncogene was a cDNA clone of the mouse cellular tumor antigen p53 which had been linked to the SV40 early-region promoter (constructed by J. Wallis). The mouse c-*myc* gene used in our earlier study had been derived from a translocated allele from the MOPC 315 plasmacytoma. This allele has not been completely sequenced, and therefore it is not known whether any mutations exist in the protein-coding region that might have influenced the activity of the c-*myc* constructs in our earlier study. In light of the controversy over the potential contribution of protein-coding mutations to c-*myc* activation in Burkitt lymphomas (2, 21, 31), murine plasmacytomas (36), and to tumor progression in avian bursal lymphomas (40), it became important to test genes that were cloned from the normal locus. However, since deregulation (constitutive expression) of the c-*myc* gene is the predominant feature of activated alleles, the normal promoter and first exon were replaced with viral promoters for both the mouse and human genes. Figure 1 shows the constructs used in these studies, in which the SV40 early-region promoter was linked to the normal mouse c-*myc* gene (SV-mmyc) or a retroviral LTR was used to replace the first exon of the normal human gene (LTR-hmyc). In the latter, an RNA splice will link the retroviral 5′ end to the protein-coding sequences in the second and third exons. Both constructs should be constitu-

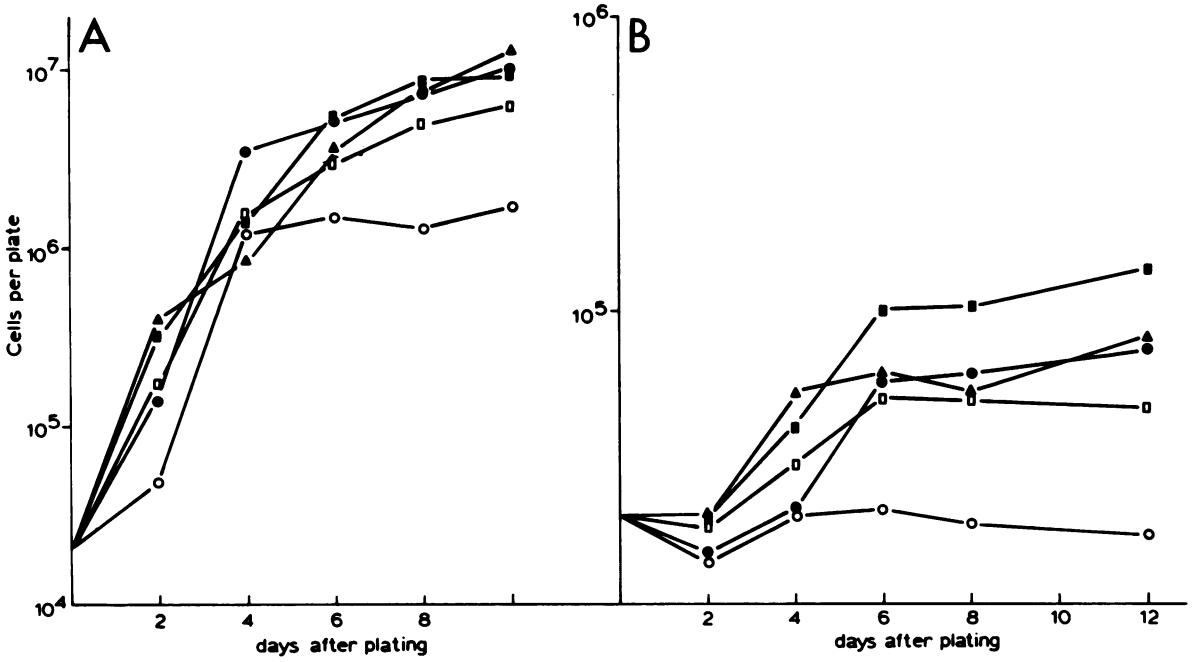

FIG. 4. Growth curves of control cells and transfectants in Dulbecco modified Eagle medium containing 10% (A) or 0.5% (B) calf serum. Cells were plated at 2×10^4 per 60-mm dish, trypsinized at intervals, and counted. Growth rates are indicated for NIH neo1 (○), NIH h*myc*1 (■), NIH m*myc*1 (▲), NIH E1a2 (●), and NIH p53.3 (□).

tively expressed in the fibroblast lines used in these studies. Details of the constructs are given in Materials and Methods.

Each of these constructs was transfected into NIH/3T3 cells with SV2neo (34) as a selectable marker in a 10:1 ratio. G418-resistant colonies were selected at random and expanded in culture for further studies. Southern blots of the transfected cells demonstrated that each line had acquired a small number of copies of the activated oncogene (Fig. 2). Photomicrographs of the cells (Fig. 3) show that, apart from a slight increase in refractility, all the lines have essentially the same morphology as control cells which had been transfected with the SV2neo gene alone.

Growth properties of isolated lines. The rate of growth in 10 and 0.5% serum media was studied for at least two lines from each oncogene transfection. Figure 4 shows growth curves for one representative from each transfection. All the oncogene-transfected lines grew at the same rate as SV2neo-transfected control NIH/3T3 cells in medium containing 10% serum (Fig. 4A). However, while the growth of the control cells stopped at confluence, all of the oncogene-transfected lines continued to grow to at least a threefold-higher cell density. In 0.5% serum, on the other hand, a much greater difference was observed between the oncogene-transfected cells and controls. While the parental NIH/3T3 cells grew very slowly, if at all, in 0.5% serum, other lines were able to grow reasonably well (Fig. 4B). Thus, all the transfected oncogenes have rendered the recipient fibroblasts independent of serum growth factors to some degree. Some differences were noted between the different oncogenes and promoters, with the LTR-driven human c-*myc* and Ad E1a genes giving the greatest low-serum growth-promoting effects, while the SVp53 gene gave the least. Different clonal isolates with the same transfected constructs gave similar results.

The cell lines were also tested for anchorage-independent growth in 0.26% agar medium (Table 1). All the oncogene-transfected lines acquired the capacity to grow to a limited extent in soft agar, although the cloning efficiencies varied. E1a- and c-*myc*-transfected cells cloned with a 5 to 8% efficiency, slightly higher than that reported previously (12). The SVp53-transfected lines gave the lowest cloning efficiency, ranging from 0.4 to 0.8% for the six lines tested. E1a- and c-*myc*-transfected lines also formed colonies larger than those observed in the p53 lines. Both the SV2neo-transfected and the untransfected NIH/3T3 lines showed negligible soft-agar clonability (<0.03%).

Tumorigenicity of oncogene-transfected lines. The cell lines transfected with the different cellular and viral oncogenes were also tested for tumorigenicity in nude mice by injecting 2×10^6 cells subcutaneously and monitoring at regular intervals thereafter for the appearance of tumors. Represen-

TABLE 1. Tumorigenicity of fibroblast lines transfected with c-*myc*, E1A, and p53 genes

Cell lines	Transfected constructs[a]	Cloning efficiency in soft agar (%)[b]	Tumorigenicity[c]	Tumor appearance (wk)
NIH/3T3		0.02	0/3	
NIH *neo*1	pSV2neo	0.02	0/3	
NIH h*myc*1	pLTRhmyc	6.0	3/3	1
NIH m*myc*1	pSVmmyc	3.5	3/3	2
NIH E1A2	p1A	4.7	3/3	1.5
NIH p53.1	p11.4	0.5	3/3	5
NIH p53.3	p11.4	0.8	3/3	4

[a] All constructs were cotransfected with pSV2neo in a 10:1 ratio.
[b] Colonies were examined 2 weeks after seeding at 5×10^4 cells per 60-mm dish in 0.26% agar.
[c] BALB/c athymic *nu/nu* mice were injected subcutaneously with 2×10^6 cells. Data are expressed as number of tumors observed after 8 weeks/number of animals injected.

tative results are shown in Table 1. All the oncogene-transfected lines were found to be tumorigenic. However, while the human *myc*-, mouse *myc*-, and E1a-transfected lines formed tumors in as little as 1 or 2 weeks, all six p53 fibroblast tumors that appeared did so only in 4 to 6 weeks. Control fibroblasts, both SV2neo-transfected and untransfected, failed to form tumors.

To demonstrate that no rearrangements of the transfected genes had taken place in the cell lines during tumor formation, cells were reestablished in culture from some of the tumors. None of the lines tested showed any observable rearrangement of genes. Figure 2 shows a representative of such an analysis in which DNAs from NIH h*myc*1 tumor tissue and tumor cells reestablished in culture were run on a gel along with DNA from the original cells used to establish the tumor. No rearrangements were detectable.

Expression of the transfected genes in the tumor lines. Northern blots of poly(A)$^+$ or total RNA from each line were used to investigate the expression of both the exogenous and endogenous genes. The NIH h*myc*1 cells contained a 2.1-kb c-*myc* transcript in addition to the expected 2.5-kb endogenous band, using a probe from the second c-*myc* exon (Fig. 5A). NIH m*myc* lines expressed, in addition to the endogenous c-*myc* transcript, a 2.25-kb RNA (data not shown). In both cases, the transfected c-*myc* genes were expressed at a level similar to that of the endogenous gene (Fig. 4A). Analysis of RNA from an E1a-transfected line (Fig. 5B) showed the presence of both the 13S and 12S RNAs, which arise from differential splicing of the primary transcript (3, 6, 20) and which are translated into 289- and 243-amino acid proteins (32). Expression of the exogenous SVp53 gene was also evident in SVp53-transfected lines (Fig. 6). In addition to the 2.0-kb endogenous p53 RNA in all three lanes, the predicted 2.5-kb exogenous transcript was evident in the NIH p53.1 and p53.3 lanes.

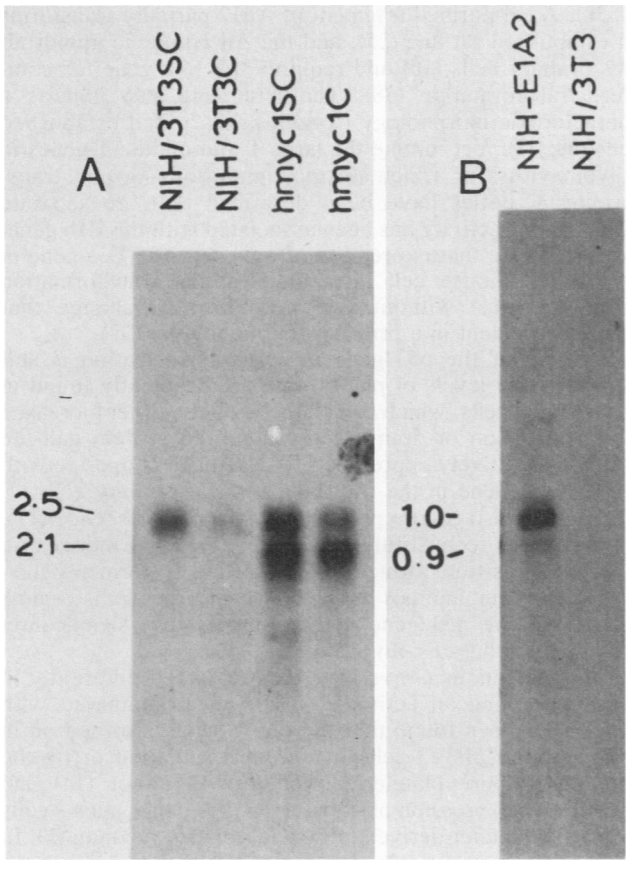

FIG. 5. Northern blot analysis of c-*myc* and E1a transfectants. (A) Poly(A)-containing RNAs (3 μg) from subconfluent (SC) and confluent (C) NIH/3T3 and NIH h*myc*1 cells were hybridized to a probe from the second c-*myc* exon (0.98-kb *Xba*I-*Sac*I [11]). The positions of the 2.5-kb endogenous c-*myc* transcript and the 2.1-kb exogenous transcript are indicated. (B) Poly(A)-containing RNAs from NIH/3T3 and NIH E1a2 cells hybridized to an E1a-specific probe. The positions of the 1.0-kb (13S) and 0.9-kb (12S) RNAs are indicated.

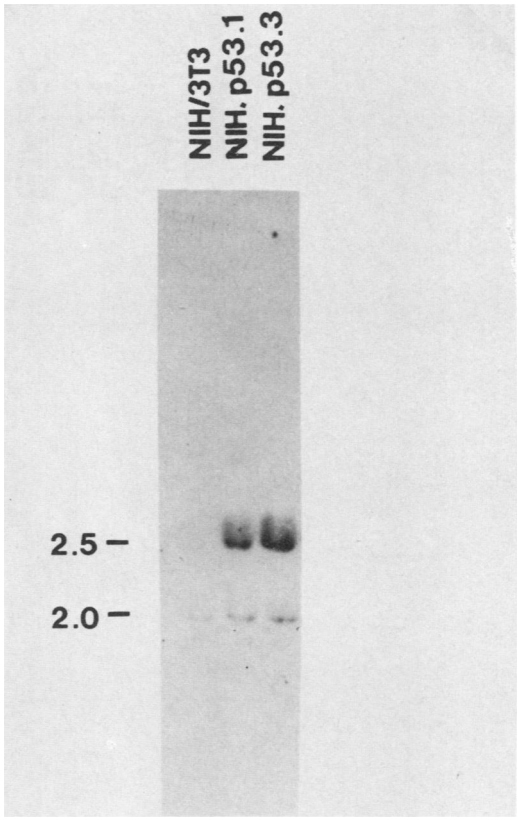

FIG. 6. Northern blot analysis of SVp53 transfectants. Total cytoplasmic RNAs (15 μg) from NIH3T3, NIH p53.1, and NIH p53.3 were hybridized to a probe from the p53 cDNA. The endogenous 2.0-kb and exogenous 2.5-kb transcripts are indicated.

Regulation of endogenous c-*myc* gene. In a previous study, it was found that the endogenous c-*myc* gene was differentially expressed in cultured cell lines that had acquired an activated c-*myc* gene, compared with the tumors that were derived from these same cells (12). In tumor cells, the endogenous c-*myc* gene was shut off, while in subconfluent cultured cells c-*myc* was expressed at the same level as in the parental lines. The NIH h*myc*1 line was used to explore this observation in a slightly different way. The cells were harvested from plates at two different densities, subconfluent and confluent, while being fed daily with fresh medium containing 10% calf serum. The density of cells at confluence with NIH h*myc*1 cells was threefold higher than could be achieved by the parental line (Fig. 4). Expression of the endogenous c-*myc* gene decreased at least threefold in the confluent cells, while expression of the transfected gene was

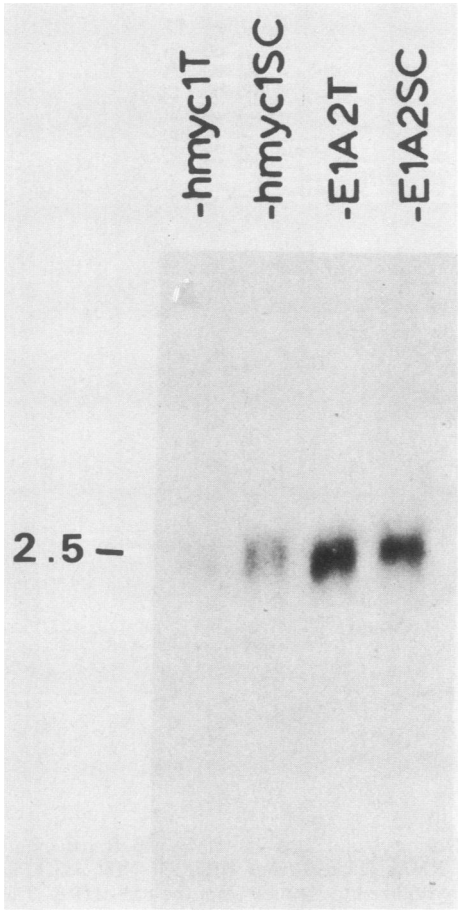

FIG. 7. Northern blot analysis of endogenous c-*myc* gene expression in NIH h*myc*1 and NIH E1a2 cells. Poly(A)-containing RNAs from NIH h*myc*1 tumors (h*myc*1T) and subconfluent cells (E1a2SC) were hybridized to a probe from the first c-*myc* exon (*Bam*HI-*Bgl*I [3]). Variation in the regulation of the endogenous 2.5-kb c-*myc* RNA is evident.

unchanged (Fig. 5A). A similar decrease was found in the NIH h*myc*1 tumors (data not shown). The parental NIH/3T3 line also showed decreased c-*myc* expression in confluent cells (Fig. 5A). Thus, contact inhibition appears to be capable of repressing c-*myc* expression even though growth factors such as platelet-derived growth factor continue to be provided.

The expression of the endogeneous c-*myc* gene was also analyzed in the E1a-transfected lines (Fig. 7). Somewhat surprisingly, the endogenous c-*myc* gene was not repressed in the E1a-induced tumors and was also expressed at a high level in the subconfluent E1a cells. We are currently examining whether deregulation of the c-*myc* gene is a consistent feature of E1a-expressing cell lines or whether it is only a feature of the transfected NIH/3T3 cells used in this study.

We also tested the DNA from the c-*myc*- and E1a-induced tumors for the presence of an activated *ras* gene, using the NIH/3T3 focus assay (24). None of the tumor DNAs were capable of inducing foci, indicating that the phenotypic changes noted here are the result of the transfected oncogenes and probably not due to secondary mutations in other cellular genes.

DISCUSSION

In an earlier study we demonstrated that an activated c-*myc* gene from a mouse plasmacytoma was capable of transforming both mouse and rat fibroblast lines (12). We have extended these studies to show that an Ad5 E1a gene, an activated mouse p53 gene, and activated normal c-*myc* genes (both mouse and human) are also capable of inducing recipient fibroblasts to acquire several properties of transformed cells, including anchorage-independent growth, a lowered serum requirement, and a tumorigenic phenotype without significant morphological alteration. Each of these cellular and viral oncogenes is localized in the nucleus and has been linked primarily with an immortalizing or establishment function. Yet we have demonstrated that each is also capable of transforming cells that have already been immortalized. This transforming function provided by the nuclear oncogenes is manifest in a less dramatic manner than that imparted by an activated *ras* gene, in that the cells are virtually unaltered morphologically and have a relatively low soft-agar cloning efficiency, unlike *ras* transformants, which clone at an efficiency of 80 to 90% in our laboratory.

Previous studies have attempted to analyze the contribution of the Ad early-region 1, E1a and E1b, toward transformation using both primary cells (10, 39) and established lines (8, 30). A gel-purified fragment of Ad12 partially transforms an established rat line (25), and the Ad E1a gene immortalizes primary cells (10) and requires the E1b gene for complete transformation (38). The E1a gene also imparts a characteristic morphology to both primary and established cells (30, 37). Yet, unlike the large T and middle T genes in polyomavirus for which distinct immortalizing and transforming activities have been described (22), no separate transforming activity has been associated with the E1b gene. We show here that expression of only the Ad5 E1a gene in established mouse cell lines induces cell transformation (tumorigenicity) without the morphological change that would be evident in a primary-cell monolayer (37).

The role of the p53 gene in cell transformation is still unclear. High levels of p53 protein are frequently found in transformed cells, which can be the result of either increased gene expression or dramatically increased protein half-life (25). Constitutively expressed p53 genes can cooperate with the *ras* oncogene in the transformation of primary cells (7, 19) and can also rescue primary cells from senescence (11). Furthermore, reconstitution of p53 expression induces an Abelson virus-transformed lymphoid line to form progressive tumors in animals (41). However, the transforming activity of the p53 gene in our studies was significantly weaker than that of c-*myc* and E1a.

The endogenous c-*myc* gene was regulated differently in cells expressing an activated c-*myc* gene compared with E1a. It has been found that the c-*myc* gene is turned on in confluent BALB/3T3 cells by serum stimulation or by the addition of pure platelet-derived growth factor (14) and constitutive expression of transfected *myc* genes allows cells to grow in platelet-derived growth factor-free medium (1). In our previous study (12), transcription of the endogenous c-*myc* gene in NIH/3T3 cells harboring a transfected c-*myc* gene was regulated as in normal cells, i.e., expression decreased in the absence of exogenous growth factors. Furthermore, expression of the endogenous c-*myc* gene also decreased in tumor tissue. In the present study, endogenous c-*myc* expression also decreased in cells maintained in a confluent state in the presence of growth factors. However, in E1a-transformed cells, the endogenous c-*myc* gene was

expressed at high levels under these same conditions. Indeed, it is possible that the phenotype of the E1a-transformed fibroblasts could be the result of this deregulation in c-*myc* expression. Further studies will be required to resolve this intriguing possibility.

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LITERATURE CITED

1. **Armelin, H. A., M. C. S. Armelin, K. Kelly, T. Stewart, P. Leder, B. H. Cochran, and C. D. Stiles.** 1984. Functional role for c-*myc* in mitogenic response to platelet-derived growth factor. Nature (London) **310**:655–660.
2. **Battey, J., C. Moulding, R. Taub, W. Murphy, T. Stewart, H. Potter, G. Lenoir, and P. Leder.** 1983. The human c-*myc* oncogene: structural consequences of translocation into the IgH locus in Burkitt lymphoma. Cell **34**:779–787.
3. **Berk, A. J., and P. A. Sharp.** 1978. Structure of the adenovirus 2 early mRNAs. Cell **14**:695–711.
4. **Bernard, O., S. Cory, S. Gerondakis, E. Webb, and J. A. Adams.** 1983. Sequence of the murine and human cellular *myc* oncogenes and two modes of *myc* transcription resulting from chromosome translocation in B-lymphoid tumours. EMBO J. **2**:2375–2383.
5. **Blair, D. G., C. S. Cooper, L. A. Eader, and G. F. Vande Woude.** 1982. New method for detecting cellular transforming genes. Science **218**:1122–1124.
6. **Chow, L. T., T. R. Broker, and J. B. Lewis.** 1979. The complex splicing patterns of RNA from the regions of Ad 2. J. Mol. Biol. **134**:265–303.
7. **Eliyahu, D., A. Raz, P. Gruss, D. Givol, and M. Oren.** 1984. Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. Nature (London) **312**:646–649.
8. **Fisher, P. B., L. E. Babiss, I. B. Weinstein, and H. S. Ginsberg.** 1982. Analysis of type 5 adenovirus transformation with a cloned rat embryo cell line (CREF). Proc. Natl. Acad. Sci. USA **79**:3527–3531.
9. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
10. **Houweling, A., P. J. van den Elsen, and A. J. van der Eb.** 1980. Partial transformation of primary rat cells by the left-most 4.5% fragment of adenovirus 5 DNA. Virology **105**:537–550.
11. **Jenkins, J. R., K. Rudge, and G. A. Currie.** 1984. Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. Nature (London) **312**:651–653.
12. **Keath, E. J., P. G. Caimi, and M. D. Cole.** 1984. Fibroblast lines expressing activated c-*myc* oncogenes are tumorigenic in nude mice and syngeneic animals. Cell **39**:339–348.
13. **Keath, E. J., A. Kelekar, and M. D. Cole.** 1984. Transcriptional activation of the c-*myc* oncogene in mouse plasmacytomas: similar RNA levels in tumor and proliferating normal cells. Cell **37**:521–528.
14. **Kelly, K., B. H. Cochran, C. D. Stiles, and P. Leder.** 1983. Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell **35**:603–610.
15. **Kriegler, M., C. F. Perez, C. Hardy, and M. Botchan.** 1984. Transformation mediated by SV40 T antigens: separation of overlapping SV40 early genes with a retroviral vector. Cell **38**:483–491.
16. **Land, H., L. Parada, and R. A. Weinberg.** 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature (London) **304**:596–602.
17. **Lawn, R. M., E. F. Fritsch, R. C. Parker, G. Blake, and T. Maniatis.** 1978. The isolation and characterization of a linked α- and β-globin gene from a cloned library of human DNA. Cell **15**:1157–1174.
18. **Mougneau, E., L. Lemieux, M. Rassoulzadegan, and F. Cuzin.** 1984. Biological activities of v-*myc* and rearranged c-*myc* oncogenes in rat fibroblast cells in culture. Proc. Natl. Acad. Sci. USA **81**:5758–5762.
19. **Parada, L. F., H. Land, R. A. Weinberg, D. Wolf, and V. Rotter.** 1984. Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. Nature (London) **312**:649–651.
20. **Perricaudet, M., G. Akusjarvi, A. Verklanes, and U. Petersson.** 1979. Structure of two spliced mRNAs from the transforming region of human subgroup C adenoviruses. Nature (London) **281**:694–696.
21. **Rabbitts, T. H., P. H. Hamlyn, and R. Baer.** 1983. Altered nucleotide sequences of a translocated c-*myc* gene in Burkitt lymphoma. Nature (London) **306**:760–765.
22. **Rassoulzadegan, M., A. Courie, A. Carr, N. Glaichenhaus, R. Kamen, and F. Cuzin.** 1982. The roles of individual polyoma virus early proteins in oncogenic transformation. Nature (London) **300**:713–718.
23. **Rassoulzadegan, M., Z. Naghashfar, A. Cowie, A. Carr, M. Grisoni, R. Kamen, and F. Cuzin.** 1983. Expression of the large T protein of polyoma virus promotes the establishment in culture of "normal" rodent fibroblast cell lines. Proc. Natl. Acad. Sci. USA **80**:4354–4358.
24. **Rave, N., R. Ckvenjakou, and H. Boedtker.** 1979. Identification of procollagen in RNAs transferred to DBM paper from formaldehyde agarose gels. Nucleic Acids Res. **6**:3559–3567.
25. **Reich, N. C., M. Oren, and A. J. Levine.** 1983. Two distinct mechanisms regulate the levels of a cellular tumor antigen, p53. Mol. Cell. Biol. **3**:2143–2150.
26. **Ruley, H. E.** 1983. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature (London) **304**:602–606.
27. **Ruley, H. E., J. F. Moomaw, and K. Maruyama.** 1984. Avian myelocytomatosis virus *myc* and adenovirus early region 1A promote the in vitro establishment of cultured primary cells, p. 481–486. *In* G. F. Vande Woude, A. J. Levine, W. C. Topp, and J. D. Watson (ed.), Cancer cells. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Shen-Ong, G. L.C., E. J. Keath, S. P. Piccoli, and M. D. Cole.** 1982. Novel *myc* oncogene RNA from abortive immunoglobulin-gene recombination in mouse plasmacytomas. Cell **31**:443–452.
29. **Shih, C., B.-Z. Shilo, M. P. Goldfarb, A. Dannenberg, and R. A. Weinberg.** 1978. Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. Proc. Natl. Acad. Sci. USA **76**:5714–5718.
30. **Shiroki, K., H. Shimogo, Y. Sawada, Y. Uemizu, and K. Fujinaga.** 1979. Incomplete transformation of rat cells by a small fragment of adenovirus 12 DNA. Virology **95**:127–136.
31. **Showe, L. C., M. Ballantine, K. Nishikura, J. Erikson, H. Kaji, and C. M. Croce.** 1985. Cloning and sequencing of a c-*myc* oncogene in a Burkitt's lymphoma cell line that is translocated to a germ-line alpha switch region. Mol. Cell. Biol. **5**:501–509.
32. **Smart, J., J. Lewis, M. Mathews, M. Harter, and C. Anderson.** 1981. Adenovirus type 2 early proteins: assignment of the early region 1A proteins synthesized in vivo and in vitro to specific mRNAs. Virology **112**:703–713.
33. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
34. **Southern, P. J., and P. Berg.** 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Gen. **1**:327–341.
35. **Spandidos, D. A., and N. M. Wilkie.** 1984. Malignant transformation of early passage rodent cells by a single mutant human oncogene. Nature (London) **310**:469–475.
36. **Stanton, L. W., P. D. Fahrlander, P. M. Tesser, and K. B. Marcu.** 1984. Nucleotide sequence comparison of normal and translocated murine c-*myc* genes. Nature (London) **310**:

423–425.
37. **van den Elsen, P. J., A. Houweling, and A. J. van der Eb.** 1983. Morphological transformation of human adenoviruses is determined to a large extent by gene products of region E1a. Virology **131**:242–246.
38. **van den Elsen, P. J., A. Houweling, and A. J. van der Eb.** 1983. Expression of region E1b of human adenoviruses in the absence of region E1a is not sufficient for complete transformation. Virology **128**:377–390.
39. **van der Eb, A. J., H. van Ormondt, P. I. Schreir, J. H. Lupker, H. Jochemsen, P. J. van den Elsen, R. J. DeLeys, C. P. van Beveren, R. Dijkema, and A. de Waard.** 1979. Structure and function of the transforming genes of human adenoviruses and SV40. Cold Spring Harbor Symp. Quant. Biol. **44**:383–399.
40. **Westaway, D., G. Payne, and H. E. Varmus.** 1984. Proviral deletions and oncogene base-substitutions in insertionally mutagenized c-*myc* alleles may contribute to the progression of avian bursal tumors. Proc. Natl. Acad. Sci. USA **81**:843–847.
41. **Wolf, D., N. Harris, and V. Rotter.** 1984. Reconstitution of p53 expression in a non-producer Ab-MuLV-transformed cell line by transfection of a functional p53 gene. Cell **38**:119–126.
42. **Zakut-Houri, R., M. Oren, B. Bienz, V. Lavie, S. Hazum, and D. Givol.** 1983. A single gene and a pseudogene for the cellular tumor antigen p53. Nature (London) **306**:594–597.