Transformation of Simian Virus 40-resistant Hamster Cells with an Adenovirus 7-Simian Virus 40 Hybrid

LEILA DIAMOND

Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

Received for publication 8 July 1967

When the hamster cell lines BHK21 and Nil-2 were infected at a multiplicity of 100 with the adenovirus 7-simian virus 40 (SV40) hybrid (strain LLE46), SV40 T antigen was induced in 0.1 to 6% of the cells during the first 96 hr postinfection, morphological changes occurred 3 to 7 weeks later, and eventually all the cells contained SV40 T antigen, but no adeno 7 T antigen. Results were similar when primary and secondary monolayer cultures of hamster embryo (HE) cells were infected with the adeno 7-SV40 hybrid, and when *primary* HE cells were infected with SV40. However, infection of BHK21, Nil-2, and *secondary* HE cells with the same multiplicity of SV40 did *not* induce SV40 T antigen or morphological transformation. This suggests that the target cells required for infection with SV40 virions, but not those required for infection with the hybrid, are lost or altered in secondary HE cultures and in the two cell lines. In most of the virus-host cell systems in which SV40 T antigen and transformation were induced, there was a decrease in the number of T antigen-positive cells after the initial infection. This was followed by a lag period of up to 2 months before the onset of a progressive increase in the number of positive cells. The beginning of the rise in T antigen production coincided with the first morphological changes.

Two hamster cell lines, BHK21 (28) and Nil-2 (11), derived from normal tissue, are susceptible to transformation by polyoma virus, but are resistant to transformation by simian virus 40 (SV40) (11, 19, 23). The mechanisms underlying this resistance may involve either the viral coat or the viral deoxyribonucleic acid (DNA). One possibility is that an early event involving the host cell and the SV40 virus particle (adsorption, penetration, or uncoating) does not take place. An alternative possibility is that, even though these early events can occur, the viral genome cannot interact with the cellular genome to yield transformation. Evidence in favor of the first possibility has been presented by Black and Rowe (3, 4), who exposed BHK21 cells to SV40 DNA.

The adenovirus 7-SV40 hybrid contains, within an adenovirus coat, a portion of the SV40 genome physically linked to adenovirus DNA (1, 15, 24, 26). The hybrid can transform cells that are susceptible to SV40-induced transformation (6, 22, 30, 31). The tumors produced by the transformed cells can resemble histologically either SV40- or adenovirus-induced tumors, or both (16, 22, 30, 31). To determine further whether failure of BHK21 and Nil-2 cells to transform after SV40 infection is due to a fault

in the early or in the late stages of virus-cell interaction, the response of these cells to infection with SV40 and with the hybrid virus has been compared.

Hamster cells transformed by SV40 contain SV40 T antigen (5), but usually no infectious SV40 can be demonstrated except by cocultivation with susceptible cells (2, 10). Hamster cells transformed by the hybrid do not yield infectious SV40 or adeno 7 virus; they contain SV40 T antigen, but may or may not contain adeno 7 T antigen (7, 22, 30, 31). Since SV40 T antigen is the marker common to cells transformed by both SV40 and the hybrid, its production was followed in the infected BHK21 and Nil-2 cells.

MATERIALS AND METHODS

Cells. Primary Syrian hamster embryo (HE) cultures were prepared from 13-day-old embryos by trypsinization with a solution of 0.25% trypsin and 0.05% ethylenediaminetetraacetate (EDTA) in phosphate-buffered saline lacking calcium and magnesium ions. The BHK21/Clone 13 cell line of hamster fibroblasts (28), obtained from M. Stoker, Institute of Virology, Glasgow, Scotland, was used within 12 to 17 passages of receipt. Nil-2, a continuous line of HE (11), was used between passages 45 and 53 when the cells were sensitive to contact inhibition of cell division and were not tumorigenic. At approximately

the 60th passage, this cell line regularly changes from an epithelioid to a fibroblastic morphology, begins to grow more rapidly, and decreases in sensitivity to contact inhibition.

The HE and Nil-2 cells were grown in Eagle's basal medium containing double-strength vitamins and amino acids. The BHK21 cells were grown in the Dulbecco-Vogt modification of Eagle's medium. Both media were supplemented with 10% calf serum and neither contained antibiotics.

Viruses. Two pools of SV40 (strain RH911) were prepared in CV-1 cells, an established line of African green monkey kidney (AGMK) (17), and had titers of $10^{8.3}$ and $10^{9.2}$ TCID₅₀/0.2 ml in primary AGMK cells. The LLE46 strain of the adenovirus 7-SV40 hybrid (15) was obtained from W. Rowe of the National Institutes of Health. A virus pool prepared in primary AGMK cells had a titer of $10^{8.2}$ TCID₅₀/0.2 ml in primary human embryonic kidney cells.

Fluorescent-antibody staining. The direct fluorescent-antibody staining test for SV40 T antigen was employed (21). Fluorescein-conjugated immune γ -globulin was purchased from Flow Laboratories, Rockville, Md. The cells were grown on cover slips (9×22 mm), fixed in acetone at 4 C for 10 min, and stained for 30 min at 37 C. The cover slips were scanned in a dark-field fluorescence microscope with a $25 \times$ objective, and the number of fluorescent nuclei per cover slip was recorded.

Complement fixation. Ten per cent cell suspensions frozen and thawed once were tested by the micro complement fixation method (27) for the presence of adeno 7 T antigen. These tests were kindly performed by R. V. Gilden of Flow Laboratories.

Hamster inoculation. After morphological transformation had occurred, 5×10^5 to 2×10^6 cells were inoculated subcutaneously into the hind leg of weanling hamsters. The tumors were fixed in Tellyesniczky's fluid, sectioned, and stained with hematoxylin and eosin.

Infection of cell cultures. Six-oz (170-ml) culture bottles and 60-mm petri plates containing cover slips were seeded with 10^6 and 2.5×10^5 cells, respectively, or, in the case of primary cells, with 3×10^6 and 7.5×10^5 cells, respectively. The cultures were infected 24 hr later with SV40 or LLE46 at a multiplicity of 100 TCID₅₀ per cell, as determined from cell counts on replicate cultures. The cell monolayers were washed twice, 0.5 ml of the appropriate virus dilution was added to each culture bottle, and two drops of the same virus dilution were placed on each cover slip. After adsorption at 37 C for 60 min, fresh medium was added to the cultures.

At daily intervals from 24 to 96 hr postinfection, cover slips were fixed for fluorescent-antibody staining. On the 4th day postinfection and twice weekly thereafter, the bottle cultures were subcultured into new bottles and into Leighton tubes containing cover slips. The cover slips were stained with fluorescent antibody 48 to 72 hr later. Duplicate cover slips were fixed in methanol and stained with May-Grünwald Giemsa. Prior to morphological transformation, the cultures, when transferred, were diluted as follows: HE, 1:2; Nil-2, 1:4 until the 58th to 60th passage, and then 1:6; and BHK21, 1:10 or 1:12.

RESULTS

Transformation of Nil-2 and BHK21 cells. Infection of Nil-2 and BHK21 cells with SV40 did not induce the production of SV40 T antigen at 24 to 96 hr postinfection (Table 1). No SV40 T antigen developed with subsequent subcultivation over a period of 2 to 3 months. During this time, the infected cultures never differed morphologically from control cultures at equivalent passage levels.

Infection of the two cell lines with LLE46 led to an initial induction of SV40 T antigen in 0.1 to 6% of the cells (Table 1). Nuclear fluorescence was observed in mononucleate and multinucleate cells; frequently, the fluorescent nuclei were multilobed. After a delay of 2 to 3 weeks, during which time the proportion of T antigen-positive cells was lower than immediately after infection, the number of positive cells in the cultures gradually began to increase. During the initial rise, there were frequently scattered clusters of positive mononucleate cells. A few weeks after the start of the second wave of T antigen-positive cells, all the cells in each culture contained SV40 T antigen. No adeno 7 T antigen could be demonstrated by complement fixation.

That the decrease and lag in T antigen production was not due to trypsinization procedures was shown by infecting replicate cover slips of Nil-2 cells with LLE46 and observing the cells over a period of 3 weeks before the first trypsinization was done. In comparison with cultures that had been trypsinized twice weekly after infection, these cells did not differ significantly in the initial decrease of T antigen-positive cells, in the length of the lag period, or in the time required for all the cells to be positive for T antigen.

The Nil-2/LLE and BHK21/LLE cell lines contained many multinucleate cells with up to 20 nuclei, each containing SV40 T antigen. These giant cells often detached from the glass, and, prior to fixation, could be seen floating in the medium. During the initial infection, and after complete transformation of the cultures, the T antigen fluorescence of the LLE-infected cells was more granular and less diffuse than that described for SV40-infected cells. In Nil-2/LLE cells, the fluorescence was very bright, and there was little variation between cells in the intensity and localization of the stain. The staining reaction in BHK21/LLE cells varied from passage to passage. At times, there were only a few brightly stained cells in each field, with most of the other cells very faintly stained. On some cover slips, there were large areas in which only the perinuclear region of the cells was stained. Despite these fluctuations, nuclear fluorescence

TABLE 1. *Development of SV40 T antigen in Nil-2 and BHK21 cells infected with SV40 and LLE 46^a*

Time postinfection	Nil-2					BHK21			
	Expt I		Expt II		Expt III	Expt I		Expt II	
	SV40	LLE	SV40	LLE	LLE	SV40	LLE	SV40	LLE
24 hr	0	0.1	0						
48 hr	0	6.2	0	5.0		0	1.0		
72 hr			0						
96 hr ^b					0.2	0	4.0	0	0.2
1 week		0.4	0	0.3	0.1		0.02	0	0
2 weeks	0	0.1	0	0.2	0		0.5		0
3 weeks	0	0.2		0	1.0		15		1.5
4 weeks		5.0		40-50 ^c	1.7	0	70-80	0	>90 ^c
5 weeks	0	4.0	0	100	1.1		100		
6 weeks		30-50 ^c				0			100
7 weeks	0	50			3.7				
8 weeks			0				100 ^c		
9 weeks		80-90				0		0	
10 weeks	0	100	0		10				
11 weeks					70-80				
12 weeks	0				100				

^a Percentage of nuclei positive for SV40 T antigen in 2×10^4 to 4×10^4 cells observed.

^b Twice-weekly trypsinization started.

^c Negative complement-fixation reaction for adenovirus 7 T antigen.

has persisted in BHK21/LLE cultures for at least 3 month after it was first observed that all the cells contained T antigen.

The morphological characteristics of Nil-2 and BHK21 cells transformed by LLE46 were typical of SV40-induced transformation (Fig. 1 and 2). Cellular alterations besides multinucleation became evident when more than 10% of the cells contained SV40 T antigen. The transformed cells were more epithelioid than the corresponding control cells and had a larger nuclear-cytoplasmic ratio. Mononucleate and multinucleate cells contained multiple, prominent nucleoli. The transformed cells grew closely packed together, although the BHK21/LLE cells frequently retained the orientation characteristic of the control cell line.

At 24 to 72 hr after plating at low cell densities ($10^4/\text{cm}^2$), the Nil-2/Control and Nil-2/LLE cells had approximately the same mean division time of 18 to 19 hr. However, the Nil-2/Control cells, even at the 80th passage, still exhibited some contact inhibition of division; at a cell density of about $10^5/\text{cm}^2$, the growth rate declined. If refed, the cells would remain attached to the glass for several weeks with reduced mitotic activity in comparison to low density cultures. In contrast, Nil-2/LLE cells continued to divide after monolayer formation but, because of overcrowding, soon detached from the glass.

One month after all cells were positive for SV40 T antigen, Nil-2/LLE and BHK21/LLE cultures were inoculated into weanling hamsters. Tumors began to appear 4 to 5 weeks later. The tumors produced by Nil-2/LLE cells were fibrosarcomas with varying degrees of anaplasia. They contained numerous multinucleate giant cells and could readily be distinguished from fibrosarcomas induced by Nil-2/Control cells (11). BHK21/LLE-induced tumors were fibrosarcomas, either with or without giant cells, and were frequently indistinguishable from tumors induced by the parent line (9). Neither Nil-2/LLE nor BHK21/LLE tumors showed areas with morphology typical of adenovirus-induced tumors. When the tumors were excised and grown in vitro, the morphology of the cells was similar to that of the cells which had been inoculated; all the cells contained SV40 T antigen.

Transformation of HE cultures. As controls for the virus-induced transformation of Nil-2 and BHK21 cells, secondary HE cultures were infected with the same multiplicities of SV40 and LLE46 and were examined in the same way for the induction of SV40 T antigen. With secondary HE cultures from three different litters, infection with LLE46 led to the development of SV40 T antigen in 0.2 to 2% of the cells at 48 to 72 hr postinfection and to morphological alterations

FIG. 1. (a) Nil-2 cells infected 13 weeks previously with LLE46. All the cells contain SV40 T antigen. (b) Nil-2/Control cells at the same passage level as the Nil-2/LLE cells in (a). May-Grünwald Giemsa stain. $\times 180$.

5 to 6 weeks later. In contrast, the SV40-infected cultures showed no initial T antigen induction, no subsequent T antigen production, and no morphological alterations after 10 weeks of subcultivation.

To investigate the unexpected finding that secondary HE cultures were as resistant to SV40-induced transformation as the Nil-2 and BHK21 cell lines, primary and secondary monolayers of two HE cultures were infected with SV40 and LLE46 (Table 2). In both *primary* cultures, T antigen was induced soon after SV40 infection. Morphological alterations became evident 4 to 6 weeks later, and the cultures eventually became fully transformed with SV40 T antigen in all the cells.

In one secondary culture infected with SV40, there was no initial T antigen induction. In the second, a few cells were positive for T antigen at 48 hr and none at 96 hr. Neither of these cultures transformed morphologically nor showed subsequent T antigen production.

In contrast to infection with SV40, infection of both the primary and secondary HE cultures

with LLE46 led to the initial induction of T antigen and to subsequent transformation of the cultures. The development of morphological alterations and of SV40 T antigen was similar in time to that observed in the SV40-transformed HE cultures, and there were no significant differences related to whether the cultures were primary or secondary monolayers when infected. Thus, there was a marked decrease between the first and second passage in the susceptibility of HE cultures to infection and transformation by SV40, but not by LLE46. That susceptibility to LLE46 was independent of passage level was shown by infecting cells of HE 123 at the 22nd passage; SV40 T antigen was induced by LLE46 in approximately 1% of these cells at 72 hr post-infection.

As was observed with Nil-2 and BHK21 cells infected with LLE46, in HE cultures in which infection with SV40 or LLE46 led to transformation, there was a lag period of varying time before the onset of the progressive increase in the number of T antigen-positive cells. In one primary culture (HE 130/LLE), there was an

FIG. 2. (a) BHK21 cells infected 9 weeks previously with LLE46. All the cells contain SV40 T antigen. (b) BHK21/Control cells at the same passage level as the BHK21/LLE cells in (a). May-Grünwald Giemsa stain. $\times 180$.

TABLE 2. Development of SV40 T antigen in hamster embryo cells infected with SV40 and LLE 46^a

Time postinfection	Primary hamster embryo				Secondary hamster embryo			
	HE 123		HE 130		HE 123		HE 130	
	SV40	LLE	SV40	LLE	SV40	LLE	SV40	LLE
48 hr	0	0	0.3	0	0	0	0.05	0
72 hr	0.01	0.01	0.7	0.04	0			
96 hr ^b	0.07	0.04			0	0.02	0	0.03
1 week	0	0	0	0.03	0	0.05	0	0
2 weeks		0.1	2.3			0.5	0	
3 weeks	0.1			0.6	0			2.0
4 weeks		5.3	3.3			25	0	
5 weeks	0.04			0.08	0			20-30
6 weeks		10	4.7			30-40	0	
7 weeks	4.3			0	0			100
8 weeks		>90	40-50			100		
9 weeks	70-80							100 ^c
10 weeks		100 ^c	100	20		100 ^c		
11 weeks	100			40-50				
12 weeks				100	0		0	

^a Percentage of nuclei positive for SV40 T antigen in 2×10^4 to 4×10^4 cells observed.
^b Twice-weekly trypsinization started.
^c Negative complement-fixation reaction for adenovirus 7 T antigen.

FIG. 3. Early morphological changes induced in hamster embryo cells by LLE46. The primary monolayer was infected 10 weeks previously (HE 130/LLE). The transformed cells contain SV40 T antigen and constitute 20% of the cell population at this time. May-Grünwald Giemsa stain. $\times 180$.

initial increase in T antigen at 3 weeks post-infection. This was followed by a second decrease, lasting about 6 weeks, before the T antigen-positive cells permanently took over the culture.

The earliest morphological changes in the HE cultures always coincided with the beginning of the rise in T antigen production. The newly transformed cells could be readily identified (Fig. 3) against the background of normal cells in the monolayer by their smaller size, increased nuclear-cytoplasmic ratio, enlarged and prominent nucleoli, loss of contact inhibition, and frequent binucleation and multinucleation. After the cultures were completely transformed and contained only T antigen-positive cells, the SV40- and LLE46-transformed cultures were essentially indistinguishable. The transformed cells were polygonal in shape, and all cultures consisted of both fibroblast-like and epithelial-like cells. The latter predominated somewhat in the LLE46-

transformed cultures, particularly in those which had been infected as primary monolayers. In the more confluent areas in the cultures, both morphological types tended to grow in criss-crossed or multilayered arrangements.

No adeno 7 antigen could be demonstrated in the HE/LLE transformed cultures. The tumors produced by these cultures were undifferentiated fibrosarcomas, similar to SV40-induced tumors, with varying numbers of giant and multinucleate cells.

DISCUSSION

The data presented here indicate that the inability of the BHK21 and Nil-2 hamster cell lines to be transformed by SV40 virus is probably due to a failure in the early stages of virus-cell interaction. Once the block is overcome by bringing the viral genome into the cells in an adenovirus coat, the genome can interact with the cells to produce SV40 T antigen and morphological alterations typical of those induced by SV40 virus. SV40 DNA can also be used to infect BHK21 cells so that they produce early SV40 T antigen (4) and acquire a "growth stimulus" and increased malignant potential (3). (The term "early T antigen" refers to antigen produced during the first 96 hr postinfection. No differences between this antigen and that observed later in morphologically transformed cells are implied.) Black and Rowe (3) observed that SV40 virus adsorbs very poorly to BHK21 cells and suggested that infection with the DNA may bypass the adsorption block.

If early T antigen production is taken as a measure of successful virus-cell interaction, then the interaction of hamster cells with the SV40 genome is extremely inefficient, whether the viral genome is introduced in an SV40 coat or in an adenovirus coat. In most virus-cell combinations in which early SV40 T antigen and eventual transformation were induced, there was a decrease in the number of T antigen-positive cells after the initial infection. This was followed by a lag period of varying time before the onset of a progressive increase in T antigen-positive cells that coincided with evidence of morphological transformation. Apparently, a period of time is required for one or more cells to become stabilized and grow out as a transformed clone(s).

In the SV40-3T3 mouse cell system where T antigen can be induced initially in nearly 100% of the cells, it has been shown that dividing cells which are not transformed can lose the ability to produce T antigen (20). Whether or not this phenomenon can account, wholly or in part, for the decrease in T antigen-positive cells in the hamster cultures is difficult to determine, since

the antigen is produced initially by only a small fraction of the cells. Black and Rowe (4) also observed a decrease in the initial number of T antigen-positive cells induced in BHK21 with SV40 DNA, but the cultures were not held to determine whether T antigen reappeared. The pattern of initial decrease and subsequent increase in T antigen-positive cells has also been observed in rodent cells infected with polyoma virus (M. Fogel, *personal communication*).

The marked difference between primary and secondary HE monolayers in susceptibility to infection with SV40 indicates that the few cells initially present which can be infected with whole SV40 virus are either lost or altered in one cell transfer. Such changes could involve cellular enzymes or cellular surface receptor sites. There is evidence in other systems for altered virus-cell interaction due to changes in cell surfaces (12, 13). The intracellular mechanisms required for SV40 T antigen production and transformation, after the viral genome is in the cell, are maintained with passage. The BHK21 and Nil-2 cell lines presumably are derived from cells that were never capable of being infected with SV40 virus or from cells that lost the potential on passage. Since both cell lines can be transformed by polyoma virus (11, 19), the target cells required for infection with this virus, as well as with LLE46, must differ from those required for infection with whole SV40 virus.

The loss, with passage, of cells that can be infected with SV40 virions seems to be unique for the Syrian hamster system. The 3T3 mouse cell line (29) and the BSC-1 (14) and CV-1 (17) AGMK lines are highly susceptible to SV40 infection. With increasing passage, human diploid cells increase, rather than decrease, in susceptibility to T antigen production (8) and transformation (18) induced by SV40.

The observation that the LLE46-transformed hamster cells did not contain adeno 7 T antigen and produced tumors resembling SV40-induced tumors is consistent with the finding of Rapp et al. (25) that the sera of hamsters bearing hybrid-induced tumors seldom contain antibody against adeno 7 T antigen unless the tumors have the histopathological characteristics of adenovirus-induced tumors.

These experiments have shown that successful transformation of hamster cells by SV40 or LLE46 can usually be predicted by observing whether infection results in SV40 T antigen production within the first 96 hr postinfection. In only one instance was early T antigen induced without eventual transformation; in every instance in which transformation occurred, there was early T antigen production. The separation

in time, by a lag period of up to 2 months, between evidence of infection (early T antigen) and the first morphological changes presents a fascinating but, as yet, unresolved problem.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service Research grants 5-R01-CA 04534-09 and 1-R01-CA 08936-01 from the National Cancer Institute and by research grant E-89 I from the American Cancer Society, Inc.

LITERATURE CITED

1. BAUM, S. G., P. R. REICH, C. J. HYBNER, W. P. ROWE, AND S. M. WEISSMAN. 1966. Biophysical evidence for linkage of adenovirus and SV40 DNA's in adenovirus 7-SV40 hybrid particles. *Proc. Natl. Acad. Sci. U.S.* **56**:1509-1515.
2. BLACK, P. H. 1966. An analysis of SV40-induced transformation of hamster kidney tissue *in vitro*. III. Persistence of SV40 viral genome in clones of transformed hamster cells. *J. Natl. Cancer Inst.* **37**:487-493.
3. BLACK, P. H., AND W. P. ROWE. 1965. Increase of malignant potential of BHK-21 cells by SV40 DNA without persistent new antigen. *Proc. Natl. Acad. Sci. U.S.* **54**:1126-1133.
4. BLACK, P. H., AND W. P. ROWE. 1965. Induction of SV40 T antigen with SV40 DNA. *Virology* **27**:436-439.
5. BLACK, P. H., W. P. ROWE, H. C. TURNER, AND R. J. HUEBNER. 1963. A specific complement-fixing antigen present in SV40 tumor and transformed cells. *Proc. Natl. Acad. Sci. U.S.* **50**:1148-1156.
6. BLACK, P. H., AND G. J. TODARO. 1965. *In vitro* transformation of hamster and human cells with the adeno 7-SV40 hybrid virus. *Proc. Natl. Acad. Sci. U.S.* **54**:374-381.
7. BLACK, P. H., AND B. J. WHITE. 1967. *In vitro* transformation by the adenovirus-SV40 hybrid viruses. II. Characteristics of the transformation of hamster cells by the adeno 2-, adeno 3-, and adeno 12-SV40 viruses. *J. Exptl. Med.* **125**:629-646.
8. CARP, R. I., AND R. V. GILDEN. 1966. A comparison of the replication cycles of simian virus 40 in human diploid and African green monkey kidney cells. *Virology* **28**:150-162.
9. DEFENDI, V., J. LEHMAN, AND P. KRAEMER. 1963. "Morphologically normal" hamster cells with malignant properties. *Virology* **19**:592-598.
10. DIAMANDOPOULOS, G. T., AND J. F. ENDERS. 1966. Comparison of the cytomorphologic characteristics of *in vitro* SV40-transformed hamster embryo cells with the histologic features of the neoplasms which they induce in the homologous host. *Am. J. Pathol.* **49**:397-417.
11. DIAMOND, L. 1967. Two spontaneously transformed cell lines derived from the same hamster embryo culture. *Intern. J. Cancer* **2**:143-152.
12. FOGEL, M., AND V. DEFENDI. 1967. Infection of muscle cultures from various species with

- oncogenic DNA viruses (SV40 and polyoma). Proc. Natl. Acad. Sci. U.S. **58**:967-973.
13. HOLLAND, J. J. 1961. Receptor affinities as major determinants of enterovirus tissue tropisms in humans. Virology **15**:312-326.
 14. HOPPS, H. E., B. C. BERNHEIM, A. NISALAK, J. H. TJO, AND J. E. SMADEL. 1963. Biologic characteristics of a continuous kidney cell line derived from the African green monkey. J. Immunol. **91**:416-424.
 15. HUEBNER, R. J., R. M. CHANOCK, B. RUBIN, AND M. J. CASEY. 1964. Induction by adenovirus type 7 of tumors in hamsters having the antigenic characteristics of SV40 virus. Proc. Natl. Acad. Sci. U.S. **52**:1333-1340.
 16. IGEL, H. J., AND P. H. BLACK. 1967. *In vitro* transformation by the adenovirus-SV40 hybrid viruses. III. Morphology of tumors induced with transformed cells. J. Exptl. Med. **125**:647-656.
 17. JENSEN, F. C., A. J. GIRARDI, R. V. GILDEN, AND H. KOPROWSKI. 1964. Infection of human and simian tissue cultures with Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S. **52**:53-59.
 18. JENSEN, F., H. KOPROWSKI, AND J. A. PONTÉN. 1963. Rapid transformation of human fibroblast cultures by simian virus 40. Proc. Natl. Acad. Sci. U.S. **50**:343-348.
 19. MACPHERSON, I., AND M. STOKER. 1962. Polyoma transformation of hamster cell clones—an investigation of genetic factors affecting cell competence. Virology **16**:147-151.
 20. OXMAN, M. N., AND P. H. BLACK. 1966. Inhibition of SV40 T antigen formation by interferon. Proc. Natl. Acad. Sci. U.S. **55**:1133-1140.
 21. POPE, J. H., AND W. P. ROWE. 1964. Detection of specific antigen in SV40-transformed cells by immunofluorescence. J. Exptl. Med. **120**:121-128.
 22. RABSON, A. S., R. A. MALMGREN, AND R. L. KIRSCHSTEIN. 1966. Induction of neoplasia *in vitro* in hamster kidney tissue by adenovirus 7-SV40 "hybrid" strain (LLE46). Proc. Soc. Exptl. Biol. Med. **121**:486-489.
 23. RAPP, F., K. S. KHERA, AND J. L. MELNICK. 1964. Resistance of BHK21 hamster cells to SV40 papovavirus. Nature **201**:1349-1350.
 24. RAPP, F., J. L. MELNICK, J. S. BUTEL, AND T. KITAHARA. 1964. The incorporation of SV40 genetic material into adenovirus 7 as measured by intranuclear synthesis of SV40 tumor antigen. Proc. Natl. Acad. Sci. U.S. **52**:1348-1352.
 25. RAPP, F., J. L. MELNICK, AND B. LEVY. 1967. Correlation of immunology and histopathology of tumors induced by defective SV40-adenovirus hybrids. Am. J. Pathol. **50**:849-859.
 26. ROWE, W. P., AND S. G. BAUM. 1964. Evidence for a possible genetic hybrid between adenovirus type 7 and SV40 viruses. Proc. Natl. Acad. Sci. U.S. **52**:1340-1347.
 27. SEVER, J. L. 1962. Application of a microtechnique to viral serological investigations. J. Immunol. **88**:320-329.
 28. STOKER, M., AND I. MACPHERSON. 1964. Syrian hamster fibroblast cell line BHK21 and its derivatives. Nature **203**:1355-1357.
 29. TODARO, G. J., H. GREEN, AND B. D. GOLDBERG. 1964. Transformation of properties of an established cell line by SV40 and polyoma virus. Proc. Natl. Acad. Sci. U.S. **51**:66-73.
 30. WELLS, S. A., JR., S. K. ORME, AND A. S. RABSON. 1966. Induction of neoplasia *in vitro* in hamster thyroid tissue by SV40 and adenovirus 7-SV40 "hybrid" (strain LLE46). Proc. Soc. Exptl. Biol. Med. **123**:507-510.
 31. WELLS, S. A., JR., R. J. WURTMAN, AND A. S. RABSON. 1966. Viral neoplastic transformation of hamster pineal cells *in vitro*: retention of enzymatic function. Science **154**:278-279.