Proc. Natl. Acad. Sci. USA
Vol. 75, No. 9, pp. 4389–4393, September 1978
Cell Biology

Simian virus 40 *A* gene function: DNA content analysis of Chinese hamster cells transformed by an early temperature-sensitive virus mutant

(cell cycle/simian virus 40 group *A* temperature-sensitive mutants/cell death/flow microfluorometry)

CHRISTINE C. ROBINSON AND JOHN M. LEHMAN*

Department of Pathology, University of Colorado Medical Center, Denver, Colorado 80262

Communicated by Hilary Koprowski, June 26, 1978

ABSTRACT **Replication of two Chinese hamster embryo cell lines transformed by an early temperature-sensitive mutant of simian virus 40, tsA58, was examined by flow microfluorometry and autoradiography of [^{3}H]thymidine-labeled cells in order to determine whether transformed cell DNA synthesis is initiated by the virus *A* gene. At the permissive temperature (37°), cells transformed by the mutant were like the wild-type virus transformants in appearance, colony-forming ability, high saturation density, and rapid replication. At the nonpermissive temperature (40.5°), the tsA58 transformed cells resembled normal embryo fibroblasts and seem to return to normal growth patterns. Although both mutant transformed cell lines at 40.5° appeared to cease growth at low saturation density, the cells did not enter a resting state, but continued to replicate. The cultures were maintained at low densities by a balance among cell replication, cell death, and sloughing of dead cells into the supernatant. These results suggest that the simian virus 40 *A* gene function effected by the tsA58 mutation does not prevent Chinese hamster embryo transformed cells from entering a resting state, although the gene may control other phenotypic characteristics of transformation.**

One approach to understanding neoplasia has been to study cells transformed by temperature-sensitive virus mutants at temperatures permissive and nonpermissive for the function of specific viral genes. In this way, the *A* gene of simian virus 40 (SV40) has been tentatively identified as the gene responsible for initiation and maintenance of transformation in mammalian cells (1–6). In the lytic system, the *A* gene initiates viral and perhaps cellular DNA synthesis (6–8). It has therefore been suggested that transformation of nonpermissive cells is maintained by the *A* gene product continuously initiating cell DNA synthesis, which would free cell replication from its normal control mechanisms (1–5, 9, 10).

If this "initiator" hypothesis is correct, cells transformed by SV40 mutants that specify a temperature-sensitive *A* gene product (tsA mutants) should not only lose the transformed phenotype at the nonpermissive temperature, but also should return to normal growth control, and arrest in the G_1 (G_0) phase of the cell cycle at confluence as normal cells do. When grown at the permissive temperature, the same cells should resume cell cycle traverse in response to the functional *A* gene. This "initiator" hypothesis was tested by analyzing the cell cycle of Chinese hamster embryo (ChH) cells transformed by wild-type (WT) SV40 and the SV40 tsA mutant, tsA58. Several independent methods of cell cycle analysis, including flow microfluorometric determination of cell DNA content, were used. The results of our studies suggest that the loss of the *A* gene function represented by the tsA58 mutation does not allow SV40-transformed ChH cells to arrest in the G_1 (G_0) phase of the cell cycle.

MATERIALS AND METHODS

Viruses and Cell Cultures. WT SV40 and the temperature-sensitive group *A* SV40 mutant, tsA58, were obtained from Peter Tegtmeyer. Virus pools were prepared and were plaque assayed on CV-1 monkey kidney cells at 33° and 40.5° (11). The presence of SV40-induced T or V antigen in infected cells was demonstrated by indirect immunofluorescence (12).

Confluent secondary cultures of normal ChH fibroblasts (12) were infected with 2–5 plaque-forming units of SV40 per cell at 37° and subcultured frequently until morphologic transformation was observed (five to six passages). This uncloned population, selected only for rapid growth on plastic, was designated ChH A58. Another embryo cell culture was similarly infected with WT or tsA58 SV40, but the cells were seeded into soft agar (13) after morphologic transformation was observed. The WT SV40-transformed clone (ChH WT-2) and the SV40 tsA58-transformed clone (ChH A58-1) were derived by picking colonies that grew in the agar at 37°. Only cultures less than 30 passages after infection were used. CV-1 cells were obtained from the American Type Culture Collection. All cultures were mycoplasma free, and were maintained in Eagle's minimal essential medium supplemented with twice the normal concentration of amino acids and vitamins, and 5% fetal bovine serum (Microbiological Associates), in a moist atmosphere of 5% CO_2/air. A single lot of serum was used in all experiments. The permissive and nonpermissive temperatures were 33° and 40.5°, respectively, for CV-1 cells, and 37° and 40.5°, respectively, for ChH cells.

Single cell suspensions for all studies were prepared by trypsinization (14). Colony formation on plastic and saturation density determinations were modified from Brugge and Butel (1). Growth in soft agar was a modification of a described technique (13). Virus was rescued from the transformed cells by fusing each cell type to CV-1 cells at 33° (15).

Cell Cycle Analysis. Single cell suspensions were fixed in 70% ethanol/30% 50 mM $MgCl_2$, and stained with mithramycin, which binds stoichiometrically to DNA (16). The flow microfluorometer FMF II at Los Alamos, NM, was used to induce and measure fluorescence of the stained cells. Data are displayed as a histogram with an abscissa representing the number of cells and an ordinate representing fluorescent intensity (channel number), which is proportional to cell DNA content.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: SV40, simian virus 40; ts, temperature-sensitive; WT, wild type; ChH cells, Chinese hamster embryo cells.

* To whom reprint requests should be addressed at Department of Pathology B216, University of Colorado Medical Center, 4200 East Ninth Avenue, Denver, CO 80262.

Autoradiography of [^{3}H]thymidine-labeled cells was performed as described (17). Cells were considered labeled if more than 20 grains over background were present over their nuclei or mitotic figures. A total of 1000 cells were scored for each time point taken.

RESULTS

Characterization of cells and virus

The appearance of the SV40-transformed and normal ChH cells was compared at 37° and 40.5°. At the permissive temperature, cultures of both tsA58 transformed cell lines contained small polygonal cells which readily formed multilayers, thus resembling the WT transformant, ChH WT-2. When the tsA58 transformants were grown at the nonpermissive temperature, however, they enlarged, flattened, and grew as monolayers which are almost indistinguishable from those formed by normal ChH cells. When 10^6 cells of ChH A58, ChH A58-1, or ChH WT-2 were injected into *nude* mice, rapidly growing, poorly differentiated fibrosarcomas were formed.

Table 1 compares the growth properties of the cells at the permissive and nonpermissive temperatures. Normal ChH cells formed a few flat, lightly stained colonies on plastic at 37°, but failed to grow on plastic at 40.5° or in soft agar at either temperature. In soft agar or on plastic, ChH WT-2 cells formed many large colonies and reached much higher saturation densities than the normal cells. At the permissive temperature, the tsA58 transformants grew to high saturation densities and formed colonies readily on plastic or in soft agar that resembled those of ChH WT-2 cells. However, at the nonpermissive temperatures, the colony-forming ability and saturation density of both ChH A58 and ChH A58-1 were low like that of normal cells.

Each cell line was fused to permissive CV-1 cells in order to determine the temperature-sensitive phenotype of the transforming virus. Virus rescued from the tsA58-transformed cells was temperature sensitive and was nearly identical to the stock SV40 tsA58 pool used for transformation, with regard to replication of viral DNA, V antigen synthesis, and plaque formation on CV-1 cells (data not shown). Virus rescued from ChH WT-2 cells resembled the WT stock virus. Each cell type was, therefore, transformed by the appropriate virus.

Thus, the characteristics used to define the transformed phenotype (cell morphology, colony formation on plastic or on soft agar, and saturation density) were quite temperature sensitive in the tsA transformed cells. The WT transformants were also somewhat temperature sensitive, as has occasionally been observed in other WT SV40-transformed cell populations (2, 3, 5, 18). The molecular mechanism of the thermal sensitivity of transformed cells is a well described, but as yet poorly understood, phenomenon (19, 20). Since the growth of the tsA58 transformed cells was always more sensitive to temperature than was WT cell growth, and at 40.5° the morphology of the tsA58 transformants returned to normal patterns as well, we therefore expected the percentage of the tsA58-transformed cells synthesizing DNA at 40.5° to roughly parallel that of normal embryo cells.

Flow microfluorometric DNA content determinations

DNA content distribution patterns were examined in two types of experiments. First, growth of the cells at 37° and 40.5° was followed as in the determinations of saturation density (see footnote, Table 1), with medium changes every other day. The second protocol was identical, except that culture fluids were not replenished during the experiment. This approach minimized temperature fluctuations and allowed the cells to exhaust the available nutrients. The results of daily DNA content analysis were identical in both types of experiments and with both tsA58 transformed cell lines, so only the patterns for ChH A58-1 cells under the more stringent growth condition, cells grown without medium replacement, are presented here.

Fig. 1 is a typical growth curve of the cells grown at 37° or 40.5° without medium replacement. Both in exponential growth (Fig. 1, day 2) and in the plateau phase (Fig. 1, day 5), flow microfluorometric analysis (Fig. 2) shows that most normal ChH embryo cells (60–80%) had a 2C DNA content and were therefore in the G_1 phase of the cell cycle. Very few (5–15%) of the cells were in the S phase. The remainder of the cells had a 4C DNA content and so were in G_2 or mitosis. In contrast to normal cells, a large fraction (30–40%) of the WT transformants at both temperatures, and the tsA transformants at 37°, were in S phase, and fewer were in G_1, both during exponential

Table 1. Growth characteristics of normal and SV40-transformed cells at permissive and nonpermissive temperatures

Cells	Temperature	Colony formation, no. colonies/100 cells		Saturation density,‡ no. cells/cm^2 ($\times 10^{-5}$)
		Plastic*	Agar†	
ChH	37°	17	0	1.0
	40.5°	0	0	0.2
	37°/40.5°	—	—	5.0
ChH WT-2	37°	65	4.0	19.0
	40.5°	4.0	0.05	7.4
	37°/40.5°	16.3	80.0	2.6
ChH A58	37°	49	2.0	10.2
	40.5°	0.02	0.01	0.8
	37°/40.5°	>1000	200	12.8
ChH A58-1	37°	37	3.0	14.2
	40.5°	0.3	0.01	2.9
	37°/40.5°	123.3	300	4.9

* Several cell concentrations were seeded into plastic dishes in Eagle's minimal essential medium with 10% fetal bovine serum. Triplicates of each dilution were incubated at 37° or 40.5°. The culture medium was changed every 3 days. After 10 days, colonies were fixed with methanol and stained with Giemsa. All colonies visible to the naked eye were scored.

† Several cell concentrations were seeded into soft agar. Triplicates of each dilution were incubated at 37° or 40.5°. After 12 days, colonies containing 20 or more cells were scored with an inverted light microscope.

‡ 3×10^4 cells per cm^2 were seeded into medium with 5% fetal bovine serum. Replicate cultures were incubated at 37° or 40.5°. The medium was changed every other day. Duplicate samples were trypsinized and counted daily in a Coulter counter. A plateau in cell counts for more than 3 days was considered to be the saturation density.

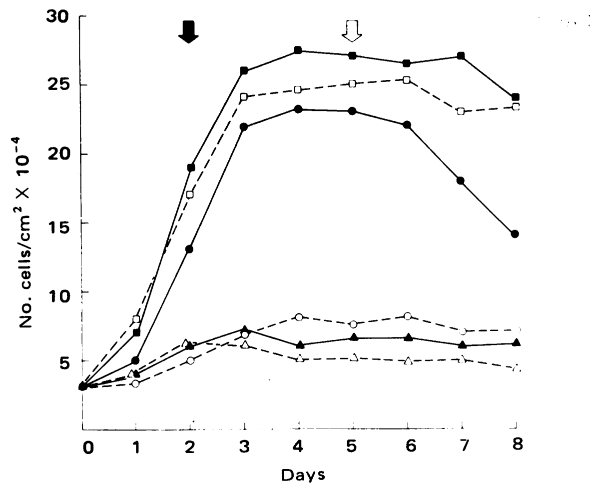

FIG. 1. Growth curves of normal and SV40-transformed ChH cells at permissive and nonpermissive temperatures. Cells ($3 \times 10^4/cm^2$) were seeded into plastic dishes in Eagle's minimal medium with 5% fetal bovine serum, and cultured at 37° or 40.5°. Medium was not changed during the experiment. Duplicate samples were trypsinized and counted daily in a Coulter counter. At the exponential phase (solid arrow) and the plateau phase (open arrow) of growth, DNA content of the cells was determined by flow microfluorometry. (—■—) ChH WT-2, 37°; (- -□- -) ChH WT-2, 40.5°; (—●—) ChH A58-1, 37°; (- -O- -) ChH A58-1, 40.5°; (—▲—) ChH, 37°, (- -△- -) ChH, 40.5°.

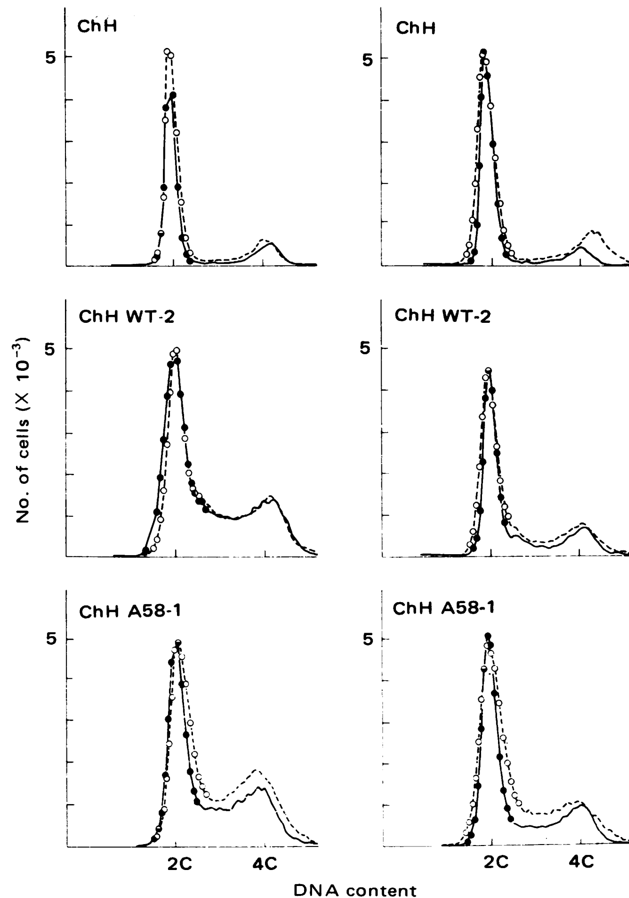

FIG. 2. Flow microfluorometric distributions of DNA content of normal and SV40-transformed ChH cells at the permissive and nonpermissive temperatures. Cell cultures are described in the legend of Fig. 1. (*Left*) Exponential phase, 2 days after subculture (Fig. 1, solid arrow). (*Right*) Plateau phase, 5 days after subculture (Fig. 1, open arrow). (—●—) Cells grown at 37°; (- -O- -) cells grown at 40.5°.

growth or in plateau phase. This pattern is consistent with the rapid growth of the cultures and the fact that even at saturation density, transformed cells can continue to traverse the cell cycle.

The "initiator" hypothesis predicted that tsA transformed cells at the nonpermissive temperature would have a DNA content distribution pattern similar to that of normal cells with very few cells in the S phase of the cell cycle, especially during the plateau phase when most apparent growth of the culture had ceased. Flow microfluorometric analysis, however, showed that the DNA content distribution pattern of the ChH A58-1 cells at 40.5° was almost identical to that of WT virus-transformed cells and ChH A58-1 cells grown at 37°. There was no evidence for a G_1 block in the tsA58 transformants at the nonpermissive temperature in stationary phase cultures. Identical results were obtained with the uncloned ChH A58 line and in experiments in which the cultures were frequently fed. Thus, despite the apparent loss of the transformed phenotype, growth of tsA58-transformed ChH cells at 40.5° does not seem to be like that of normal cells.

Autoradiography

There are several possible explanations for the large percentage of tsA58 transformed cells in the S phase of the cell cycle at 40.5°, despite the apparent plateau in growth of the culture. The first possibility, that progress of the cells through all phases of the cell cycle is blocked, would suggest that the *A* gene is involved in traverse of transformed cells through the entire cell cycle. The second possibility is that once a certain saturation density is reached, cell replication is balanced by cell death. To distinguish between these two possibilities, the cultures depicted in Fig. 1 were exposed to [^{3}H]thymidine for 1 hr or for 48 hr at the exponential and plateau phases of growth, and autoradiography was performed. The results are presented in Table 2.

During exponential growth, many normal ChH cells were in mitosis or were incorporating [^{3}H]thymidine over their nuclei. These fractions decreased, however, as the cells became confluent. At both temperatures and in both growth phases, many ChH WT-2 cells incorporated thymidine or were in mitosis, as expected for transformed cells with little density-dependent inhibition of growth.

The labeling pattern of ChH A58-1 cells at 37° resembled that of the WT virus transformant. The large fraction of ChH A58-1 cells with labeled nuclei at the plateau phase of growth at 40.5° confirms the flow microfluorometric finding that many of the cells were actively synthesizing DNA even when the growth of the culture appeared to cease. Most of these cells seemed to move freely through S phase and into mitosis because almost all ChH A58-1 mitotic figures were heavily labeled in a 48-hr pulse at 40.5°. Identical results were obtained with the uncloned ChH A58 cell line. To determine whether cell-to-cell contact was necessary to allow the cells to enter a resting state, we grew the tsA58 transformed cells at 37° until confluent and then shifted them to the nonpermissive temperature. Regardless of whether or not the medium was replaced at the time of shift up, the cells at 37° continued to replicate, while the 40.5° cultures ceased apparent growth within 24 hr (data not shown). Autoradiography and flow microfluorometric analysis, however, showed that all of the cells at 40.5° were still actively traversing the cell cycle.

Finally, time-lapse cinematography of the tsA58 transformed cells was performed. At confluence at 40.5°, many cells were

Table 2. Autoradiography of [^{3}H]thymidine-labeled normal and SV40 transformed ChH cells at permissive and nonpermissive temperatures

Cells	Temperature	% labeled nuclei*		% labeled mitoses†		Mitotic index‡	
		Exponential	Plateau	Exponential	Plateau	Exponential	Plateau
ChH	37°	16	7	100	100	0.6	0.3
	40.5°	13	5	100	100	0.9	0.5
ChH WT-2	37°	52	28	100	100	4.5	3.3
	40.5°	56	34	99	94	6.2	5.7
ChH A58-1	37°	52	33	99	98	5.6	4.6
	40.5°	48	41	100	96	6.3	5.1

Cell cultures are described in the legend of Fig. 1. Exponential phase is 2 days after subculture; plateau phase is 5 days after subculture.

* Percent of cells with grains over nucleus after a 1-hr pulse of [^{3}H]thymidine, 0.5 μCi/ml.

† Percent of mitotic figures with grains after a 48-hr pulse of [^{3}H]thymidine, 0.1 μCi/ml.

‡ Mitotic figures per 100 cells.

released into the supernatant, while the remaining adherent cells formed a monolayer. The cell loss seemed to take place primarily during interphase and reflects cell death, since most of the supernatant cells took up trypan blue and could not be replated at 37° or 40.5°. The adherent cells, however, remained viable, as shown by trypan blue exclusion, and could be subcultured indefinitely at the nonpermissive temperature. If the cultures were shifted from 40.5° to 37° in the absence of fresh medium, the number of adherent cells began to increase within 24 hr (unpublished data). As expected for cells that continue to replicate at both temperatures, after the shift down there was very little change in the DNA content distribution patterns or in the percent of cells incorporating [^{3}H]thymidine.

These results demonstrate that tsA58-transformed Chinese hamster cells do not return to normal growth patterns at 40.5° and do not arrest in the G_1 phase of the cell cycle at confluence.

DISCUSSION

The results of this study are consistent with previous reports that the SV40 *A* gene maintains many of the properties of the transformed phenotype (1–5). However, flow microfluorometric analysis of cell DNA content showed that under conditions where normal embryo cells enter a resting state, the tsA58 transformants at 40.5° continue to replicate. The low density of the mutant virus transformed cell cultures at 40.5° is simply maintained by a balance among cell proliferation, cell death, and sloughing of dead cells into the culture medium, rather than representing a return of the cells to normal growth control. Since cell replication can be dissociated from other phenotypic characteristics of transformation, it appears that in this system loss of phenotypic characteristics of transformation cannot be explained by arrest of the cells in the G_1 (G_0) phase of the cell cycle.

This unusual growth control mechanism was observed in tsA58 transformed cell cultures derived from two different batches of embryos, in both cloned and uncloned populations. As recently described for transformation by polyoma virus (21), we observed a considerable difference in the degree of temperature sensitivity exhibited by the two SV40 tsA58 transformed cell lines, depending on the method used to select the cells. The growth control mechanism of the two cell lines at the nonpermissive temperature, however, seems identical. Similar growth patterns have also been observed for certain WT SV40-transformed revertants of mouse 3T3 cells (22), SV40-transformed 3T3 cells grown in low serum (23) or treated with cyclic AMP (24), and temperature-sensitive mutants of chemically transformed cells (25). We do not yet know why these cells show the same growth pattern as ChH A58-1 cells, or whether the same regulatory mechanism will be found in other cell types transformed by tsA58.

In contrast to our results, Chinese hamster lung cells transformed by another SV40 tsA strain, tsA239, appeared to cease replication and failed to incorporate [^{3}H]thymidine into DNA at the nonpermissive temperature in depleted medium (10). This behavior was interpreted as a return of the cells to normal growth control in the absence of the *A* gene function.

We cannot completely explain the difference between these two sets of results. Subtle differences between lung fibroblasts and whole embryo fibroblasts, such as higher nonpermissive temperature requirements, were considered. Even at 41.5°, when the WT transformants began to die and normal ChH cells enter a resting state, tsA58 transformed cells were still incapable of arresting in G_1 (unpublished data). Another possible explanation may be the slightly higher temperature used in our study to select for transformants. Others also found it necessary to select tsA transformants of certain cell types at 37° rather than at 33° (J. Brugge, personal communication). Since our tsA transformants display the full transformed phenotype at 33° as well as at 37° (unpublished data) and closely resemble the lines selected by others at 33° (1, 3), we consider this explanation for the different results unlikely. Transformation of the cells by a revertant in the tsA58 stock was ruled out by the rescue of temperature-sensitive virus from the ChH A58 cells. The viruses used in the two studies are similar, since the mutations of both viruses map within the same restriction endonuclease fragment (26).

The major difference between these two studies may arise in the methods used for the cell cycle analysis. Flow microfluorometric analysis of cell DNA content, now an established tool for studying the cell cycle (27), is probably preferable to methods relying solely on [^{3}H]thymidine uptake in systems where virus infection or temperature alone may interfere with pool sizes or transport of DNA precursors (unpublished data), thymidine kinase activity (28), or cell viability (16, 29). Indeed, the use of [^{3}H]thymidine, even short pulses, of low specific activity, can actually induce a G_2 block in Chinese hamster cells (30). Flow microfluorometry can easily distinguish between a resting state in the G_1 phase of the cell cycle and such a G_2 block (16).

An insight into the *A* gene function in transformed Chinese hamster cells may be obtained by learning why tsA58 transformed ChH cells replicate and die at the nonpermissive temperature. Since other early SV40 mutants have been reported to excise from the host cell genome at the nonpermissive temperature (31), cell death resulting from virus reactivation at the nonpermissive temperature was considered, but no significant

amounts of virus have been found. The fact that the tsA58 transformants can be subcultured indefinitely at 40.5° suggests that the cells are not simply senescing as normal fibroblasts in cell culture ultimately do. Loss of the *A* gene function might reduce the ability of some of the cells to survive the stress of the elevated temperature, followed by compensatory "normal" repopulation by the surviving cells. But normal ChH embryo cells enter a resting state and do not die at the nonpermissive temperature. Neither do the WT virus transformed cells die at 40.5°, which also suggests a different method of growth regulation in the presence of a WT *A* gene product, and argues against a nonspecific hyperthermic effect as the cause of cell death.

Burstin and Basilico have described temperature-sensitive cell mutants that enter G_0 at the nonpermissive temperature (32). These mutants die when they are infected with polyoma virus at the nonpermissive temperature, probably because polyoma induces the cells to traverse the S phase of the cell cycle in an unbalanced way. Could death of the tsA58 transformed cells at 40.5° be due to a similar phenomenon, the conflict between two signals within the cell, one from a signal constantly initiating cell DNA synthesis and the other a temperature-sensitive function specifying a return to a more normal phenotype? With the recent discovery of other proteins encoded by the SV40 early region (33), our results may be explained by separate viral functions stimulating cell DNA synthesis and maintaining transformation. Since the experiments presented here and the results of Dubbs *et al.* (34) show that SV40 tsA transformed cells continue replication at the nonpermissive temperature, we believe that the "initiator" hypothesis for the function of the *A* gene product in the maintenance of transformation must be reevaluated.

We acknowledge helpful discussions with Drs. L. Scott Cram, James Jett, and Douglas Swartzendruber. This investigation was supported in part by Grants CA-16030 and CA-15823 from the National Institutes of Health, and by the United States Energy Research and Development Administration, Los Alamos Scientific Laboratory.

1. Brugge, J. S. & Butel, J. S. (1975) *J. Virol.* **15**, 619–635.
2. Kimura, G. & Itagaki, A. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 673–677.
3. Martin, R. G. & Chou, J. Y. (1975) *J. Virol.* **15**, 599–612.
4. Osborn, M. & Weber, K. (1975) *J. Virol.* **15**, 636–644.
5. Tegtmeyer, P. (1975) *J. Virol.* **15**, 613–618.
6. Tegtmeyer, P. (1972) *J. Virol.* **10**, 591–598.
7. Chou, J. Y., Avila, J. & Martin, R. G. (1974) *J. Virol.* **14**, 116–124.
8. Chou, J. Y. & Martin, R. G. (1975) *J. Virol.* **15**, 145–150.
9. Levine, A. J. (1973) in *Perspectives in Virology*, ed. Pollard, M. (Academic, New York), Vol. 8, pp. 61–79.
10. Martin, R. G. & Stein, S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1655–1659.
11. Carp, R.I. & Gilden, R. V. (1966) *Virology* **28**, 150–162.
12. Defendi, V. & Lehman, J. M. (1965) *J. Cell Comp. Physiol.* **66**, 351–410.
13. Macpherson, I. & Montagnier, L. (1964) *Virology* **23**, 291–294.
14. Tobey, R. A., Crissman, H. A. & Kraemer, P. M. (1972) *J. Cell Biol.* **54**, 638–645.
15. Davidson, R. L., O'Malley, K. A. & Wheeler, T. B. (1976) *Somat. Cell Genet.* **2**, 271–278.
16. Tobey, R. A. & Crissman, H. A. (1975) *Exp. Cell Res.* **93**, 235–239.
17. Lehman, J. M. & Defendi, V. (1970) *J. Virol.* **6**, 738–749.
18. Anderson, F. & Martin, R. (1976) *J. Cell Physiol.* **88**, 65–76.
19. Kase, K. & Hahn, G. M. (1975) *Nature (London)* **255**, 228–229.
20. Mondovi, B., Agro, A., Rolilio, G., Strom, R., Moricca, G. & Fanelli, A. (1976) *Eur. J. Cancer* **5**, 137–146.
21. Seif, R. & Cuzin, F. (1977) *J. Virol.* **24**, 721–728.
22. Jainchill, J. L. & Todaro, G. J. (1970) *Exp. Cell Res.* **59**, 137–146.
23. Bartholomew, J. C., Yokota, H. & Ross, P. (1975) *J. Cell Physiol.* **88**, 277–286.
24. Paul, D. (1972) *Nature (London) New Biol.* **240**, 179–181.
25. Yamaguchi, N. & Weinstein, B. I. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 214–218.
26. Lai, C.-J. & Nathans, D. (1975) *Virology* **66**, 70–81.
27. Gelfant, S. (1977) *Cancer Res.* **37**, 3845–3862.
28. Postee, E. H. & Levine, A. J. (1976) *Virology* **73**, 206–215.
29. Schulman, N. & Hall, E. J. (1974) *Radiology* **113**, 209–211.
30. Ehmann, V. K., Williams, J. R., Nagel, W. A., Brown, J. A., Belli, J. A. & Jett, J. T. (1975) *Nature (London)* **258**, 633–636.
31. Lockwood, M. P., Rothschild, H. & Wilson, M. (1977) *Nature (London)* **266**, 720–722.
32. Burstin, S. J. & Basilico, C. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2540–2544.
33. Crawford, L. V., Cole, C. N., Smith, A. E., Paucha, E., Tegtmeyer, P., Rundell, K. & Berg, P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 117–121.
34. Dubbs, D. R., Trkula, D. & Kit, S. (1978) *Somat. Cell Genet.* **4**, 95–110.