JOURNAL OF VIROLOGY, June 1969, p. 686-692

Vol. 5, No. 6
Printed in U.S.A.

Deoxyribonucleic Acid Replication in Simian Virus 40-Infected Cells

III. Comparison of Simian Virus 40 Lytic Infection in Three Different Monkey Kidney Cell Lines

EARL RITZI AND ARNOLD J. LEVINE

Department of Biochemistry, Princeton University, Princeton, New Jersey 08540

Received for publication 25 February 1970

A comparative study of simian virus 40 (SV40) lytic infection in three different monkey cell lines is described. The results demonstrate that viral deoxyribonucleic acid (DNA) synthesis and infectious virus production begin some 10 to 20 hr earlier in CV-1 cells and primary African green monkey kidney (AGMK) cells than in BSC-1 cells. Induction of cellular DNA synthesis by SV40 was observed in CV-1 and AGMK cells but not with BSC-1 cells. Excision of large molecular weight cellular DNA to smaller fragments was easily detectable late in infection of AGMK cells. Little or no excision was observed at comparable times after infection of CV-1 and BSC-1 cells. The different kinds of responses of these three monkey cell lines during SV40 lytic infection suggest the involvement of cellular functions in the virus-directed induction of cellular DNA synthesis and the excision of this DNA from the genome.

Purified preparations of polyoma virus grown in primary mouse kidney cells contain a variable proportion of virions that enclose cellular deoxyribonucleic acid (DNA) instead of viral DNA (17, 24). These viral particles have been termed pseudovirions (17). Studies with polyoma virus have indicated that several virus-directed events must occur in the formation of pseudovirions. Thus, it appears that induction of cellular DNA synthesis is required for the excision or fragmentation of cellular DNA to a size suitable for packaging into virions (2, 3). If this is correct, then at least three major steps in the production of pseudovirions can be recognized: (i) induction of cellular DNA synthesis, (ii) excision or fragmentation of cellular DNA to a size suitable for enclosure into virions, and (iii) packaging of this DNA by the viral capsids and assembly into virions.

Purified preparations of simian virus 40 (SV40) grown in primary African green monkey kidney (AGMK) cells contain SV40 pseudovirions (16). Few or no SV40 psudovirions could be detected in purified SV40 preparations grown in BSC-1 or CV-1 cells (16). In an effort to determine why SV40 pseudovirions were not produced in these permanent cell lines, a comparative study of the events occurring during lytic infection of AGMK, BSC-1, and CV-1 cells was undertaken.

Experiments presented in this paper demonstrate that, after infection of CV-1 and AGMK cells with SV40, the rate of cellular DNA synthesis increases 8- to 10-fold. On the other hand, no induction of cellular DNA synthesis was detected in BSC-1 cells. These data are in good agreement with previously published reports (6, 9, 13, 22, 23). Between 13 and 23% of the AGMK cell DNA was found to be excised to small molecular weight fragments by 96 hr after infection. In contrast to this, excision or fragmentation of cellular DNA did not occur in BSC-1 cells and was observed at a much reduced level (1 to 2%), if at all, in CV-1 cells. These data explain why SV40 pseudovirions are formed in AGMK cells and not in CV-1 or BSC-1 cells. In addition, they yield information bearing on the mechanism involved in induction and excision of cellular DNA after infection with SV40.

MATERIALS AND METHODS

Virus. The SV40 large-plaque mutant (15) was employed in the experiments where virus was grown in primary AGMK cells. The wild-type strain of SV40 (15) was used when virus was grown in BSC-1 or CV-1 cells. The results presented in this paper are independent of whether one uses the SV40 large-plaque mutant or wild-type strain. The large-plaque virus was employed with AGMK cells because higher virus titers are obtained with this combination (18,

20). This virus grows very poorly in BSC-1 and CV-1 cells (18, 20).

Tissue culture. Primary AGMK cells (Flow Laboratories, Inc., Rockville, Md.) were cultured in plastic petri dishes (100 by 20 mm; Falcon Plastics, Los Angeles, Calif.) in Dulbecco's modified Eagles medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% calf serum. BSC-1 cells (from R. Pollock) and CV-1 cells (from H. S. Ginsberg) were grown in the same fashion.

Infectivity assay. Virus stocks were prepared as previously described (16). Infectious virus was titrated on monolayer cultures of AGMK cells or BSC-1 cells by use of a plaque assay procedure (7, 14).

DNA-DNA hybridization. Purified SV40 supercoiled DNA was obtained as described previously (16). DNA-DNA hybridization was performed by use of the procedure of Denhardt (5) as modified by Aloni, Winocour, and Sachs (1).

Measurement of DNA synthesis. The rate of viral DNA synthesis was measured by the addition of ^{3}H-thymidine (14.2 to 17.9 c/mmole; New England Nuclear Corp., Boston, Mass.) to infected cell cultures. After a 1-hr exposure to the isotope, the cells were washed twice with ice cold phosphate-buffered saline (PBS) and lysed with 0.6% sodium dodecylsulfate (SDS) in 0.01 M sodium phosphate buffer (*p*H 7.2), 0.15 M NaCl, and 0.01 M disodium ethylenediaminetetraacetic acid (EDTA). Viral DNA was then fractionated away from cellular DNA by the Hirt procedure (10). A portion of the 1 M NaCl-SDS soluble fraction (small molecular weight DNA fraction) was sedimented through a 5 to 20% linear sucrose gradient for 3 hr at 40,000 rev/min in an SW50.1 rotor. The ^{3}H-labeled component that sedimented at 20 to 21*S* in this gradient was used to quantitate the rate of viral DNA synthesis. The quantity of ^{3}H-thymidine found in the 1 M NaCl-SDS precipitate fraction (large molecular weight DNA) was employed to determine the rate of cellular DNA synthesis. Only that fraction of the ^{3}H-label that was trichloroacetic acid-precipitable and resistant to alkaline degradation (0.3 M KOH, 37 C for 18 hr) was used to measure the rate of cellular DNA synthesis.

After a 1-hr exposure of SV40-infected AGMK cells with ^{3}H-thymidine, over 90% of the radioactive label in the 1 M NaCl-SDS soluble fraction is viral specific (15). In addition, more than 90% of the ^{3}H-label found in the 1 M NaCl-SDS precipitate fraction is cellular DNA (15).

Measurement of the reduction in large molecular weight cellular DNA to small molecular weight molecules. Actively growing cell cultures (2×10^5 to 4×10^5 cells plated into 100 × 20 mm petri dishes) were labeled with ^{3}H-thymidine (1 μc/ml) for two to three generations. These cells were then washed with unlabeled medium and allowed to continue growing in fresh medium without isotope. At the end of this time, the cells became confluent monolayers and were infected with SV40. At 95 to 96 hr after infection, the cells were harvested, and the intracellular DNA was fractionated into a 1 M NaCl-SDS soluble and precipitable fraction as described by Hirt (10). For uninfected cells, 97 to 98% of the ^{3}H label was present in the 1 M NaCl-SDS precipitate fraction. An increase in the ^{3}H-labeled cellular DNA in the 1 M NaCl-SDS soluble fraction of infected cells (above the 2 to 3% observed in uninfected cells) was taken as an indication that cellular DNA was broken down to a lower molecular weight form. The available evidence indicates that the 1 M NaCl-SDS fractionation procedure separates DNA molecules on the basis of their molecular weight (10). The DNA labeled with ^{3}H-thymidine prior to the infection and found in the 1 M NaCl-SDS soluble fraction after infection with SV40 is cellular DNA. This will be demonstrated in the Results section.

Centrifugation techniques and isotope measurements. Sucrose gradient centrifugation was performed as previously described (16). The details of each sedimentation run are given in the figure legends. Radioactive samples were prepared and counted in a Beckman liquid scintillation counter as described previously (16).

RESULTS

Kinetics of infectious virus production in AGMK and BSC-1 cell lines. To compare the events that occur in AGMK, BSC-1, and CV-1 cell lines during lytic infection, it was necessary to determine the kinetics of infectious virus production in these cell lines. Cultures of AGMK and BSC-1 cells were infected with SV40, and at various times thereafter the cells were harvested, sonically treated, and titrated for infectious virus. A comparison of the production of infectious SV40 in AGMK and BSC-1 cells is presented in Fig. 1. In AGMK cells an increase in the level of infectious virus could be detected between 20 and 24 hr after infection. On the other hand, the first indication of increased levels of virus in BSC-1 cells occurred at 36 to 45 hr after infection. The one-step growth curve for infectious SV40 production in CV-1 cells was very similar to that observed for AGMK cells (12, 15). The kinetics of infectious virus production in BSC-1 cells are in good agreement with previously published data (6).

Kinetics of viral DNA synthesis in AGMK, BSC-1, and CV-1 cell lines. AGMK, BSC-1, and CV-1 cell cultures were infected with SV40 and pulse-labeled with ^{3}H-thymidine for a 1-hr period at various times after infection. The kinetics of viral DNA synthesis was measured as described in Materials and Methods, and these data are presented in Fig. 2. SV40-specific DNA was first synthesized in detectable quantities in AGMK and CV-1 cells between 12 and 20 hr after infection. Viral DNA synthesis in BSC-1 cells was first observed at 30 to 38 hr after infection. These results are in agreement with previously published reports (6, 13, 15).

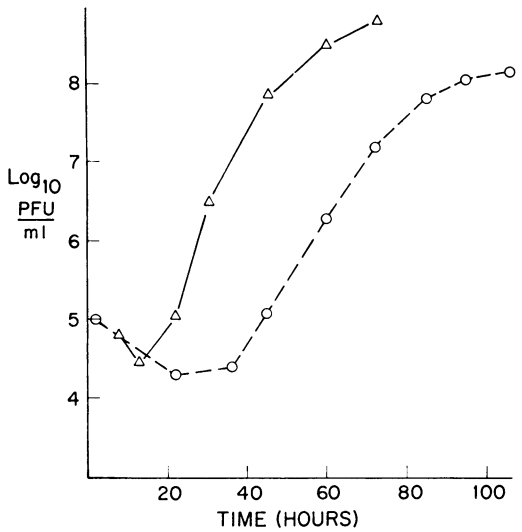

FIG. 1. *Kinetics of SV40 infectious virus production in AGMK and BSC-1 cells. AGMK and BSC-1 cells were infected with SV40 (0 hr; multiplicity of infection, 50 to 100 plaque-forming units/cell), and at the times indicated above a culture was harvested, sonically treated and titrated for infectious virus. Symbols: △, SV40 grown in AGMK cells; ○, SV40 grown in BSC-1 cells. SV40 grown in CV-1 cells usually gave yields of virus intermediate between AGMK and BSC-1 cells.*

Kinetics of virus induced cellular DNA synthesis in AGMK, BSC-1, and CV-1 cell lines. The three cell lines under investigation were infected with SV40 and pulse-labeled with ^{3}H-thymidine at various times after infection. The rate of synthesis of cellular DNA (1 M NaCl-SDS precipitate fraction) was then measured (Fig. 3). The kinetics of induction of cellular DNA synthesis in AGMK and CV-1 cells were similar and in good agreement with published results from other laboraties (9, 13, 22, 23). Induction of cellular DNA in these cells began at about the same time as viral DNA synthesis. Little or no induction of cellular DNA synthesis was observed in BSC-1 cells. This is in agreement with results first reported by Gershon, Sachs, and Winocour (6).

Comparing the incorporation of ^{3}H-thymidine into cellular DNA in infected and uninfected cells may suffer from problems in pool sizes, kinase levels, or permeability differences. To eliminate this problem, the total quantity of cellular DNA (1 M NaCl-SDS precipitate) present before and after infection was measured colorimetrically by means of the diphenylamine test (4). The total amount of AGMK cellular DNA (4×10^7 cells) increased from 460 to 895 μg by 65 hr after infection. This is an increase of 1.95-fold. The level of CV-1 cellular DNA (2×10^7 cells) increased from 266 to 497 μg at 65 hr after infection. This is a 1.86-fold increase in the amount of cellular DNA. In contrast to these data, the level of BSC-1 cellular DNA (2×10^7 cells) increased from 209 to 239 μg by 96 hr after infection. This is only a 1.1-fold increase in the quantity of cellular DNA. These data are consistent with the results presented in Fig. 3.

Conversion of high-molecular-weight cellular DNA to low-molecular-weight fragments. After infection of primary mouse kidney cells with polyoma virus, the cellular DNA is cut into small molecular weight fragments (2, 3). This process was investigated in AGMK, CV-1, and BSC-1 cells infected with SV40. The reduction in molecular weight of cellular DNA after infection was measured by appearance of cellular DNA, labeled with ^{3}H-thymidine prior to infection, in the 1 M NaCl-SDS soluble fraction. The experiments demonstrating the fragmentation of cellular DNA (to a small molecular weight) after infection are presented in Table 1. At 96 hr after infection of AGMK cells, between 13 and 23% of

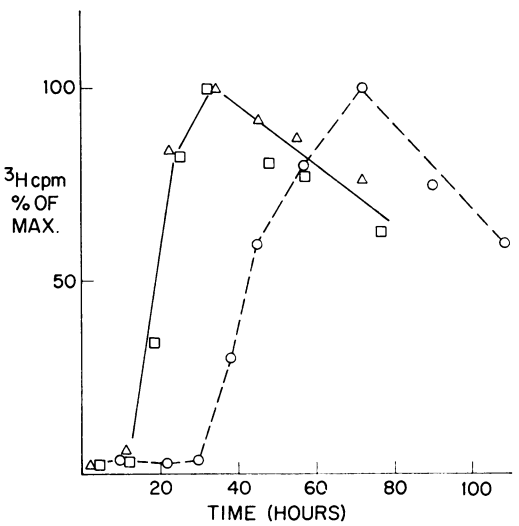

FIG. 2. *Kinetics of viral DNA synthesis in AGMK, BSC-1, and CV-1 cells. Each of the three cell lines was infected with SV40 (0 hr), and a culture was pulse-labeled with ^{3}H-thymidine (1 μc/ml) for 1 hr at the times indicated above. A sample of the 1 M NaCl-SDS soluble fraction was sedimented through a sucrose gradient, and the ^{3}H-labeled DNA that sedimented at 20 to 21S was used to determine the quantity of viral DNA synthesized during the 1-hr pulse. SV40 DNA produced in AGMK cells (△), BSC-1 cells (○), and CV-1 cells (□). The 100% values for AGMK cells, CV-1 cells, and BSC-1 cells were 2,690, 1,800, 1,300 counts/min.*

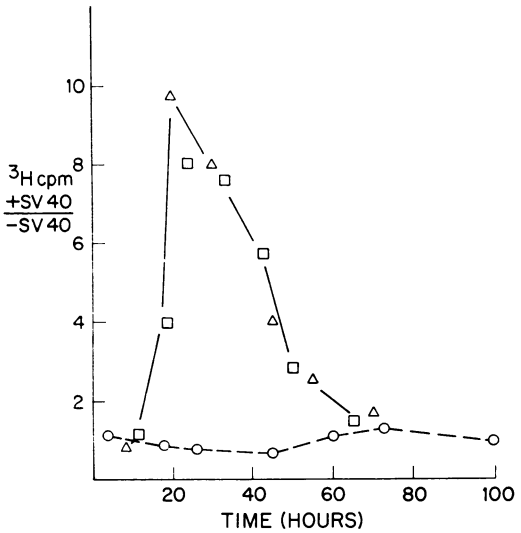

FIG. 3. *Kinetics of cellular DNA synthesis in AGMK, BSC-1, and CV-1 cells after infection with SV40. The experimental procedure is as described in Fig. 2 except that a sample of the 1 M NaCl-SDS precipitate fraction was employed to determine the quantity of cellular DNA synthesized in a 1-hr pulse-labeling period. Cellular DNA synthesis in infected AGMK (△), BSC-1 (○), and CV-1 (□). The ordinate is expressed as the ratio of ^{3}H-thymidine incorporated in a 1-hr pulse in infected cells divided by the level of incorporation in uninfected cells. The values obtained with uninfected AGMK, CV-1, and BSC-1 cells (the 1.0 value) were 1,400, 1,850, and 820 counts/min, respectively. The level of incorporation of ^{3}H-thymidine into growing cells was 8- to 20-fold higher than these resting-cell levels. Depleted medium (21) was employed in all monolayer experiments.*

the cellular DNA behaved as if it had a decreased molecular weight. About 1 to 2% of the CV-1 cell DNA and little or none of the BSC-1 cell DNA behaved as low molecular weight DNA fragments at 96 hr after infection.

Two lines of evidence demonstrate that the DNA labeled with ^{3}H-thymidine prior to infection and found in the 1 M NaCl-SDS soluble fraction after infection is cellular DNA. The DNA-DNA hybridization experiments presented in Table 2 demonstrate that the DNA labeled prior to infection and isolated in the 1 M NaCl-SDS supernatant after infection hybridizes like cellular DNA. In support of this conclusion, it was found that 98% of this DNA was fully denaturable into single strands after heating to 100 C for 4 min (0.01 M NaCl, 0.01 M tris (hydroxymethyl)aminomethane - hydrochloride, *p*H 7.2). The production of single-stranded DNA by this heat treatment, which is a property of cellular DNA and not closed circular viral DNA, was tested by benzoylated-naphoylated diethylaminoethyl cellulose chromatography as described previously (15, 16).

Sedimentation profiles of cellular DNA found in the 1 M NaCl-SDS soluble fraction late in infection. To determine the size distribution of cellular DNA found in the 1 M NaCl-SDS soluble fraction 96 hr after infection of AGMK cells, a sample of this fraction was sedimented through a 5 to 20% linear sucrose gradient. The 1 M NaCl-SDS soluble fraction from a culture labeled with ^{3}H-thymidine prior to a mock infection was sedimented in a second sucrose gradient as an uninfected control. The sedimentation profiles of the 1 M NaCl-SDS soluble fraction from an SV40-infected and an uninfected AGMK cell culture are presented in Fig. 4. As can be seen, there was a heterogeneous distribution of sizes of cellular DNA present in the small molecular weight DNA fraction (1 M NaCl-SDS soluble).

TABLE 1. *Fragmentation of large molecular weight cellular DNA after infection with SV40*

Cell line	Expt. no.	Percentage of total cellular DNA in 1 M NaCl-SDS soluble fraction[a] at			
		95-96 hr		125 hr	
		+SV40	−SV40	+SV40	−SV40
AGMK...	1	15.1	2.2	—	—
	2	27.5	4.7	—	—
CV-1.....	1	5.3	2.7	—	—
	2	4.8	3.4	—	—
BSC-1....	1	3.1	2.9	—	—
	2	2.8	2.7	3.8	3.2

[a] These data demonstrate the percentage of the total cellular DNA that is found as small molecular weight fragments in AGMK, CV-1, or BSC-1 cells at 96 or 125 hr after infection.

TABLE 2. *DNA-DNA hybridization of viral, cellular, and prelabeled 1 M NaCl-SDS soluble DNA*

Source of DNA	Total counts/min	Per cent bound to filter[a]		
		Viral	AGMK	No DNA[b]
SV40.....	7,100	24.5	2.0	0.46
AGMK.....	81,000	1.9	17.9	0.33
Prelabeled AGMK in 1 M NaCl-SDS soluble fraction.....	25,500	1.3	14.0	0.41

[a] Viral and AGMK DNA, 15 μg/filter.

[b] Backgrounds not subtracted from per cent bound to filters.

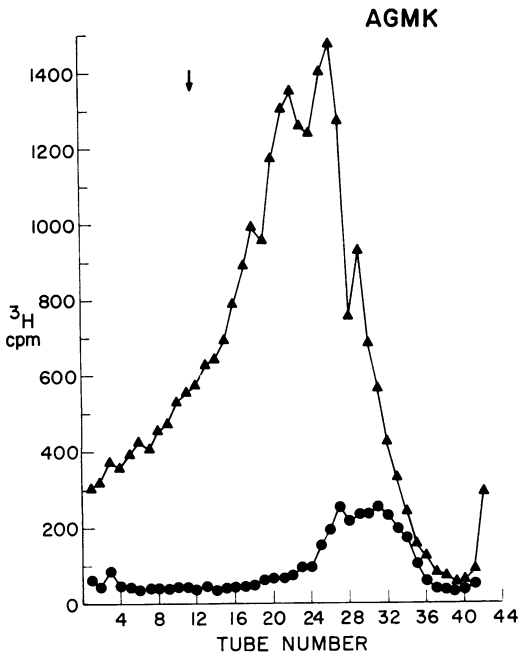

FIG. 4. *Sedimentation profile of AGMK cellular DNA in the 1 M NaCl-SDS soluble fraction. AGMK cells were labeled prior to infection with ^{3}H-thymidine. One-half of the cell cultures were infected with SV40, and 96 hr later the infected and uninfected cultures were harvested as described in Materials and Methods. A sample of the 1 M NaCl-SDS soluble fraction was sedimented through a 5 to 20% linear sucrose gradient for 3.5 hr at 45,000 rev/min in an SW50.1 rotor. Symbols: ▲, infected AGMK cells; ●, uninfected AGMK cells. The arrow indicates the position of a ^{32}P-labeled SV40 sedimentation marker (21S).*

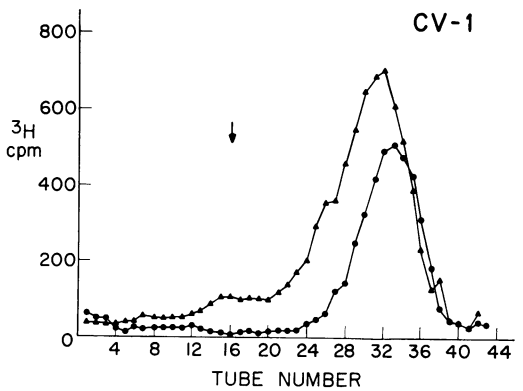

FIG. 5. *Sedimentation profile of CV-1 cellular DNA in the 1 M NaCl-SDS soluble fraction. The details of the experimental procedure are given in Fig. 4. Symbols: ▲, infected CV-1 cells; ●, uninfected CV-1 cells. The arrow indicates the position in the gradient of a ^{32}P-labeled SV40 sedimentation marker.*

The majority of DNA sedimented in the region of 11 to 22S in the gradient.

Figure 5 compares the sedimentation profile of the 1 M NaCl-SDS soluble cellular DNA from infected and uninfected CV-1 cells. These data suggest that a small proportion of cellular DNA is altered in its size after infection and sediments between 14 and 21S. It is clear, however, that the levels of fragmented cellular DNA are much reduced when compared with AGMK cells. Figure 6 presents the results of a similar experiment performed with BSC-1 cells. Little or no difference in the size or quantity of cellular DNA in the 1 M NaCl-SDS soluble fraction was observed between infected and uninfected BSC-1 cells.

DISCUSSION

A summary of the results obtained in this study are presented in Table 3. The different responses of these three cell lines during SV40 lytic infection may help unravel some of the mechanisms involved in induction and excision of cellular DNA.

SV40 lytic infection of AGMK and CV-1 cells, but not BSC-1 cells, results in the induction of cellular DNA synthesis (6, 9, 13, 22, 23). These facts indicate that AGMK and CV-1 cells

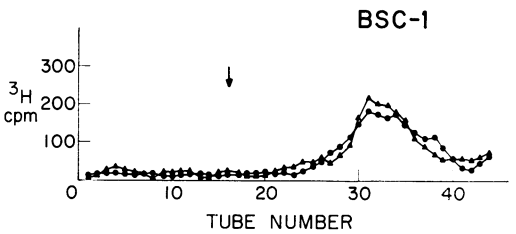

FIG. 6. *Sedimentation profile of BSC-1 cellular DNA in the 1 M NaCl-SDS soluble fraction. The details of the experimental design are given in Fig. 4. Symbols: ▲, infected BSC-1 cells; ●, uninfected BSC-1 cells. The arrow indicates the position in the gradient of a ^{32}P-labeled SV40 sedimentation marker.*

TABLE 3. *Summary of data for AGMK, CV-1, and BSC-1 cells*

Cell line	Time of appearance of virus-specific		Cellular DNA		Pseudovirions
	DNA	Infectious SV40	Induction	Excision	
	hr	*hr*			
AGMK	15–20	20–24	+	+ (13–23%)	+
CV-1	15–20	20–24	+	∓ (1–2%)	−
BSC-1	30–38	36–45	−	−	−

contain a cellular component that is either altered or not present in BSC-1 cells, and that is required for the virus-directed induction of cellular DNA. It is possible that this cellular component was altered or lost during the extablishment of BSC-1 cells as a permanent monkey cell line (11). DNA preparations obtained from isolated nucleoli of CV-1 cells contain a dense satellite band (1.712 g/cc in CsCl) not present in BSC-1 cells (J. J. Maio, *personal communication*). This may be relevant to the problem of induction of cellular DNA synthesis because the first DNA species synthesized after SV40 infection of AGMK monolayers is localized in the nucleolar region of the cell nucleus (8). A similar result has been observed with baby mouse kidney cell monolayers infected with polyoma virus (19).

Monolayer cultures of BSC-1 cells can be stimulated to synthesize cellular DNA at an increased rate (8- to 14-fold) by the addition of fresh serum to the medium (21; Levine, *unpublished results*). These data imply that serum-mediated induction of cellular DNA synthesis is different from virus-directed induction of cellular DNA synthesis. It is important to point out that one cannot test for an increased rate of cellular DNA synthesis unless there is a marked decline in the rate of DNA synthesis as the cells approach a monolayer condition. The cell lines used for these experiments were continuously passed at a light cell density, avoiding the appearance of cells that could overgrow the monolayer (21). In the experiments presented in this paper, the AGMK, CV-1, and BSC-1 cell lines showed an 8- to 20-fold decrease in the rate of DNA synthesis after they formed a monolayer.

The fact that CV-1 and BSC-1 cells possess little or no capacity to excise cellular DNA after infection indicates that at least a part of this process in AGMK cells is effected by a cell-specific component(s). This component(s) apparently degrades only cellular DNA that has been replicated after infection (2, 3). This suggests that the virus-directed induction of cellular DNA synthesis yields a species of DNA that is different from the cellular DNA made in a "normal" round of DNA replication.

Pseudovirions made in AGMK cells contain DNA (cellular) that sediments between 11 and 15*S*, or about 1.5×10^6 to 3.5×10^6 daltons (16). The DNA excised from the genome during infection is very heterogeneous, sedimenting throughout the sucrose gradient. The majority of this DNA sediments between 11 and 22*S* (about 1.5×10^6 to 9.0×10^6 daltons). The size selectivity of pseudovirion DNA would appear to be a reflection of the quantity of cellular DNA that can be fit into the SV40 virus particle [about 3.2×10^6 daltons (15)].

Finally, it should be pointed out that the results presented in this paper demonstrate why SV40 pseudovirons are found in virus preparations made in AGMK cells and not in those virus stocks prepared in CV-1 or BSC-1 cells (16).

ACKNOWLEDGMENTS

The excellent technical assistance of Angelika K. Teresky is gratefully acknowledged.

This research was supported by Public Health Service grant CA 11049-01 from the National Cancer Institute and by grant E-591 from the American Cancer Society.

LITERATURE CITED

1. Aloni, Y., E. Winocour, and L. Sachs. 1968. Characterization of the simian virus 40-specific RNA in virus-yielding and transformed cells. J. Mol. Biol. 31:415–429.
2. Ben-Porat, T., C. Cato, and A. S. Kaplan. 1966. Unstable DNA synthesized by polyoma virus-infected cells. Virology 30:74–81.
3. Ben-Porat, T., and A. S. Kaplan. 1967. Correlation between replication and degradation of cellular DNA in polyoma virus-infected cells. Virology 32:457–464.
4. Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colormetric determination of deoxyribonucleic acid. Biochem. J. 62:315–322.
5. Dendardt, D. 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophy. Res. Commun. 23:641–646.
6. Gershon, D., L. Sachs, and E. Winocour. 1966. The induction of cellular DNA synthesis by simian virus 40 in contact-inhibited and X-irradiated cells. Proc. Nat. Acad. Sci. U.S.A. 56:918–925.
7. Gilead, Z., and H. S. Ginsberg. 1965. Characterization of a tumorlike antigen in type 12 and type 18 adenovirus-infected cells. J. Bacteriol. 90:120–125.
8. Granboulan, N., and P. Tournier. 1965. Horaire et Localisation de la synthèses des acids nucleques pendant la phase d'eclipse du virus SV40. Ann. Inst. Pasteur (Paris) 109:837–854.
9. Hatanaka, M., and R. Dulbecco. 1966. Induction of DNA synthesis by SV40. Proc. Nat. Acad. Sci. U.S.A. 56:736–740.
10. Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. 26:365–369.
11. Hopps, H. E., B. C. Bernheim, A. Nisalak, J. H. Tjio, and J. E. Smadel. 1963. Biologic characteristics of a continuous kidney cell line derived from the African green monkey. J. Immunol. 91:416–424.
12. Kit, S. 1967. Enzyme inductions in cell cultures during productive and abortive infections by papovavirus SV40, p. 495–525. *In* J. S. Colter and W. Paranchych (ed.), The molecular biology of viruses. Academic Press Inc., New York.
13. Kit, S., R. A. de Torres, D. R. Dubbs, and M. L. Salvi. 1967. Induction of cellular deoxyribonucleic acid synthesis by simian virus 40. J. Virol. 1:738–746.
14. Levine, A. J., and H. S. Ginsberg. 1967. Mechanism by which fiber antigen inhibits multiplication of type 5 adenovirus. J. Virol. 1:747–757.
15. Levine, A. J., H. S. Kang, and F. E. Billheimer. 1970. DNA replication in SV40 infected cells. I. Analysis of replicating SV40 DNA. J. Mol. Biol., *in press*.
16. Levine, A. J., and A. K. Teresky. 1970. Deoxyribonucleic Acid replication in simian virus 40-infected cells. II. Detection and characterization of simian virus 40 pseudovirions. J. Virol. 5:451–457.
17. Michel, M. R., B. Hirt, and R. Weil. 1967. Mouse cellular

DNA enclosed in polyoma viral capsids (pseudovirions). Proc. Nat. Acad. Sci. U.S.A. 58:1381–1388.
18. Ozer, H. L., and K. K. Takemoto. 1969. Site of host restriction of simian virus 40 mutants in an established African green monkey kidney cell line. J. Virol. 4:408–415.
19. Smith, B. J. 1970. Light satellite-band DNA in mouse cells infected with polyoma virus. J. Mol. Biol. 47:101–106.
20. Takemoto, K. K., G. J. Todaro, and K. Habel. 1968. Recovery of SV40 virus with genetic markers of original inducing virus from SV40 transformed mouse cells. Virology 35:1–8.
21. Todaro, G., Y. Matsuya, S. Bloom, A. Robbins, and H. Green. 1967. Stimulation of RNA synthesis and cell division in resting cells by a factor present in serum. *In* V. Defendi and M. Stoker (ed.), Growth regulating substances for animal cells in culture. Monograph 7, p. 87–101. Wistar Inst. Press, Philadelphia.
22. Werchau, H., H. Westphal, G. Maass, and R. Haas. 1966. Untersuchunger uber den Nucleinsauerestaff-wechsel von Affenierengewebe-Kullurzellen nach Inpktion nut SV40. II. Einfluss der Infelstion auf den DNS-Sloffwechsel der Zelle. Arch. Gesamte Virusforsch. 19:351–360.
23. Westphal, H., H. Werchau, G. Maass, and R. Haas. 1966. Untersunchungen uber den Nucleinsauerestaff-wechsel von Affenierengewebe-Kulturzellen nach Infektion mit SV40 I. Darstelhing and Chromalographie der SV40-DNS. Arch. Gesamte-Virusforsch. 19:342–350.
24. Winocour, E. 1969. Some aspects of the interaction between polyoma virus and cell DNA. Advan. Virus Res. 14:153.