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Deoxyribonucleic Acid Replication in Simian Virus 40-Infected Cells

II. Detection and Characterization of Simian Virus 40 Pseudovirions

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Purified simian virus 40 (SV40) virions, grown in primary African green monkey kidney cells labeled prior to infection with ^{3}H-thymidine, contain a variable quantity of ^{3}H-labeled deoxyribonucleic acid (DNA). This DNA is resistant to deoxyribonuclease, sediments at 250*S*, and is enclosed in a particle that can be precipitated with SV40-specific antiserum. DNA-DNA hybridization experiments demonstrate that this ^{3}H-labeled component in purified SV40 virions is cellular DNA. When this ^{3}H-labeled DNA is released from purified virus with sodium dodecyl sulfate, it has an average sedimentation constant of 14*S*. Sedimentation through neutral and alkaline sucrose gradients shows that this 14*S* DNA is composed of a collection of different sizes of DNA molecules that sediment between 11 and 15*S*. As a result of this size heterogeneity, SV40 virions containing cellular DNA (pseudovirions) have a variable DNA to capsid protein ratio and exhibit a spectrum of buoyant densities in a CsCl equilibrium gradient. Pseudovirions are enriched, relative to true virions, on the lighter density side of infectious SV40 virus banded to equilibrium in a CsCl gradient. Little or no cellular DNA was found in purified SV40 virus preparations grown in BSC-1 or CV-1 cells.

Infection of primary mouse kidney cells with polyoma virus results in an induction of cellular deoxyribonucleic acid (DNA) synthesis (8, 16, 19). Some of this cellular DNA is then cut into molecular low-weight fragments (3) and packaged into polyoma virions (13, 18). Highly purified preparations of polyoma virus thus contain linear mouse DNA enclosed in polyoma capsid proteins (13, 18). These viral particles have been termed polyoma pseudovirions (13).

In this paper, experiments are presented that demonstrate cellular DNA in purified simian virus 40 (SV40) virus preparations grown in primary African green monkey kidney (AGMK) cells. This cellular DNA behaves as if it were enclosed in viral particles on the basis of its buoyant density in CsCl, sedimentation rate, resistance to deoxyribonuclease, and ability to be precipitated with SV40 antiserum. These particles have been termed SV40 pseudovirions. Few or no pseudovirions could be detected in purified SV40 virus preparations grown in BSC-1 or CV-1 cells.

MATERIALS AND METHODS

Virus. The SV40 large-plaque mutant was employed when the virus was grown in primary AGMK cells. This virus was kindly supplied by V. Defendi. The SV40 wild-type strain, kindly supplied by J. Vinograd, was employed when the virus was grown in BSC-1 or CV-1 cells.

Tissue culture. Monolayer cultures of primary AGMK cells (Flow Laboratories) were grown on plastic petri dishes (100 by 20 mm) in Dulbecco's modified Eagle medium (Grand Island Biological Co.) supplemented with 10% calf serum. Monolayer cultures of BSC-1 cells (obtained from R. Pollock) and CV-1 cells (obtained from H. S. Ginsberg) were grown in the same fashion.

Preparation of virus stocks. Monolayer cultures of primary AGMK cells were infected with SV40 large-plaque virus (BSC-1 and CV-1 with SV40 wild-type virus) at an input multiplicity of 25 to 100 plaque-forming units (PFU) per cell in 2 ml of Dulbecco's medium supplemented with 2% calf serum. After 2 hr of adsorption at 36 C, an additional 10 ml of the same medium was added. At 4 to 6 days, the cells were scraped from the petri dish surface with a rubber policeman, centrifuged out of suspension, and resuspended in one-tenth volume of 0.15 M NaCl in 0.01 M sodium phosphate buffer (PBS) at *p*H 7.5. The cells were then sonically treated for 5 min with a Branson Sonifier at 0 to 4 C. These stocks were titered and stored at −20 C.

Infectivity assay. Infectious virus was titered on monolayers of AGMK cells or BSC-1 cells by using the plaque assay procedure (9, 12).

Purification of the virus. SV40 was purified by a modification of the procedure first described by Black, Crawford, and Crawford (4). Infected cells were prepared and sonically treated as described above. These sonic-treated materials were cleared by centrifugation at 12,000 × *g* for 30 min. The pellets were treated for 30 min at 37 C with 1% sodium deoxycholate and recentrifuged at 12,000 × *g* for 30 min. The deoxycholate supernatant fluid was added to the sonically treated supernatant fluid and centrifuged for 3 hr at 60,000 × *g*. The pellet was resuspended in 1 ml of PBS and usually left overnight at 4 C. The particulate debris was then centrifuged out of suspension at 12,000 × *g* for 30 min. The supernatant fluid was treated with ribonuclease (10 μg/ml) for 30 min at 37 C and was then adjusted to a buoyant density of 1.32 g/ml by the addition of solid cesium chloride; this solution was centrifuged to equilibrium (115,000 × *g* for 20 to 24 hr). The virus was collected, and the CsCl density equilibrium centrifugation was repeated. The recovery of virus at the end of this procedure was about 70%. The purity of the virus will be dealt with below.

DNA-DNA hybridization. DNA-DNA hybridization was performed by the procedure first described by Denhardt (7) and modified by Aloni, Winocour, and Sachs (1).

Purified viral DNA. Closed circular supercoiled viral DNA was obtained by the following procedure. Infected cells were washed with cold PBS and lysed by the addition of 1 ml of 0.6% sodium dodecyl sulfate (SDS) in 0.01 M tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 6.5) and 0.01 M disodium ethylenediaminetetraacetic acid (EDTA). Viral DNA was then fractionated from high-molecular-weight cellular DNA by the procedure described by Hirt (10). The low-molecular-weight DNA fraction (viral DNA) was extracted with redistilled phenol saturated with 1 M NaCl, Tris-hydrochloride buffer (0.01 M, *p*H 7.2), and 0.01 M EDTA. The phenol was removed by dialysis against 0.01 M NaCl, 0.01 M Tris-hydrochloride (*p*H 7.2), and 0.01 M EDTA. The phenol-extracted DNA was heated for 4 min at 100 C and rapidly chilled in an ice bath. The NaCl concentration of this solution was adjusted to 0.3 M and the solution was put on a benzoylated-naphthoylated diethylaminoethyl (DEAE)-cellulose column (1 by 1 to 2 cm). Closed circular (nondenaturable) DNA was eluted with 1 M NaCl. The denatured DNA remained on the column. This procedure is a modification of that first described by Kamano and Sinsheimer (11).

Centrifugation techniques. Sucrose gradient centrifugation was performed by sedimenting 0.2 ml of the sample through a 5 to 20% linear sucrose gradient (1 M NaCl, 0.01 M Tris-hydrochloride at *p*H 7.2, and 0.01 M EDTA) for the time and speeds indicated in each figure. Samples were collected through a hole punctured in the bottom of the tube onto Whatman 3MM filter pads (2.3 cm). The filter pads were dried and washed twice with cold 10% trichloroacetic acid and once with cold acetone. The dried filters were placed in scintillation vials, a toluene-spectrafluor (Amersham-Searle) mixture was added, and the samples were counted in a Beckman liquid scintillation counter.

Immunological precipitation of purified virus. Purified SV40 virus in PBS plus 0.5% bovine serum albumin was incubated with rabbit anti-SV40 (Grand Island Biological Co.) for 1 hr at 37 C. At this time, sheep anti-rabbit serum was added and incubation at 37 C was continued for an additional 1.5 hr. The precipitate that formed was centrifuged out of suspension at 1,000 × *g* for 30 min at 4 C and the supernatant fluid was removed. The precipitate was washed twice with cold 10% trichloroacetic acid and then filtered onto a Whatman GFA filter pad. This was counted in the liquid scintillation counter as described previously.

RESULTS

Purification of SV40. To demonstrate the existence of cellular DNA wrapped in viral particles, it was important to show that free cellular DNA does not contaminate the purified virus preparations. To this end, three monolayer cultures of primary AGMK cells were infected with SV40. Fifteen hours later ^{3}H-thymidine (10 μc/ml) was added to each culture. At the same time, three growing uninfected cultures of primary AGMK cells were labeled with ^{32}P (inorganic phosphate, 100 μc/ml). The infected cultures (^{3}H-labeled) and the uninfected cultures (^{32}P-labeled) were harvested at 96 hr after infection and mixed together. The virus was then purified as described in the previous section.

Figure 1 presents the profile of radioactivity and infectivity obtained in the second CsCl equilibrium centrifugation step of the purification. A single peak of ^{3}H-labeled material is observed which coincides with the infectivity. A total of 93,000 trichloroacetic acid-precipitable ^{32}P-counts per minute was added to the crude lysate of infected cells at the beginning of the purification. Approximately 215 ^{32}P-counts per minute remained under the virus peak at the end of the purification. These data indicate that purified virus preparations contain less than 1% nucleic acid (and phospholipid) contamination

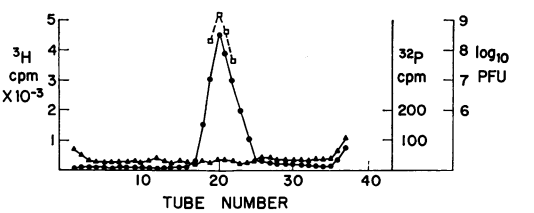

FIG. 1. *CsCl equilibrium gradient of purified SV40. ^{32}P-labeled uninfected cells were mixed with ^{3}H-thymidine-labeled infected cells and the virus was purified as described in Materials and Methods. Centrifugation was in an SW39 rotor for 24 hr at 115,000 × g. Symbols: ●, ^{3}H counts/min; ▲, ^{32}P counts/min; □, PFU/fraction.*

when added as an uninfected cell lysate. It is assumed that infected cell-free nucleic acids would behave in a similar manner.

Detection of DNA labeled before infection in purified SV40 virus preparations. Three growing cultures each of primary AGMK cells, CV-1 cells, and BSC-1 cells were labeled with ^{3}H-thymidine (10 μc/ml) for 2 days and chased with unlabeled thymidine for 2 additional days. At the end of this time, all of the cultures became monolayers and were infected with SV40. At 4 days after infection, the AGMK and CV-1 cells were harvested and at 6 days after infection the BSC-1 cells were harvested. The virus obtained from each cell culture was purified as previously described. The purified virus was broken open with 0.6% SDS. The lysed virus particles were dialyzed against 1.0 M NaCl, 0.01 M Tris-hydrochloride buffer at *p*H 7.2, and 0.01 M EDTA in the cold. The cesium dodecyl sulfate precipitate was removed by centrifugation. A 0.2-ml sample of the purified viral lysate was sedimented in a 5 to 20% linear sucrose gradient. Before centrifugation, a 21*S*, ^{32}P-labeled supercoiled viral DNA sedimentation marker was added to the sample. Fractions of the gradient were collected and counted as described.

Figure 2 presents the sedimentation profile of radioactivity observed in this experiment. SV40 virus grown in primary AGMK cells prelabeled with ^{3}H-thymidine contained a component that sedimented at about 14*S* with respect to the ^{32}P-labeled 21*S* viral DNA marker. Little or no ^{3}H-labeled DNA cosedimented with the supercoiled viral DNA. Purified virus grown in CV-1 cells contained little, if any, of the 14*S* ^{3}H-labeled component (Fig. 2B). In purified virus grown in BSC-1 cells, no detectable ^{3}H-label above the background level was observed.

The 14*S* component observed in purified virus preparations made in AGMK cells was DNA. This was demonstrated by the fact that it was trichloroacetic acid-precipitable and resistant to alkaline degradation; 95% of this ^{3}H-label was rendered acid-soluble by treatment with pancreatic deoxyribonuclease (10 μg/ml for 1 hr at 37 C).

Evidence that the DNA labeled before infection and found in purified SV40 virus preparations is enclosed in viral capsids. Purified virus preparations grown in AGMK cells that had been labeled with ^{3}H-thymidine before infection were prepared. The ^{3}H-labeled component that had a buoyant density of 1.32 g/ml in the CsCl equilibrium gradient was collected in PBS containing 0.5% bovine serum albumin and dialyzed against 1.0 M NaCl in 0.01 M Tris-hydrochloride buffer at *p*H 7.2. The virus was then sedimented

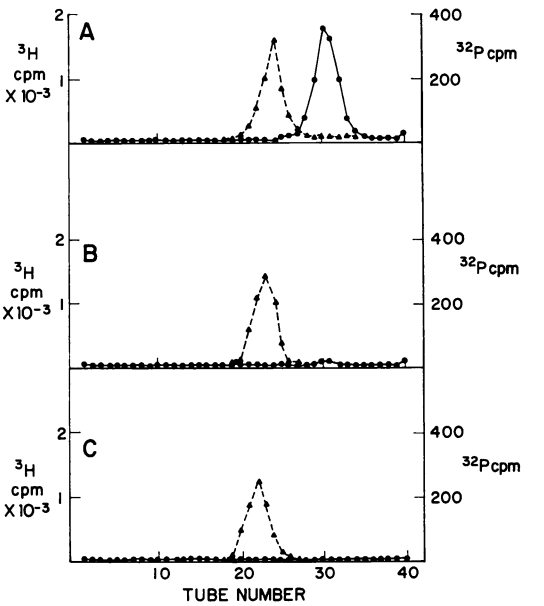

FIG. 2. *Sucrose gradient velocity centrifugation of cellular DNA isolated from purified SV40. SV40 was grown in AGMK cells (A), CV-1 cells (B), and BSC-1 cells (C) that were labeled with ^{3}H-thymidine before infection. The virus was purified, broken open with SDS, and sedimented in an SW50.1 rotor for 3 hr at 130,000 × g. Symbols: ●, ^{3}H counts/min; ▲, ^{32}P counts/min added as a 21S, SV40 DNA, sedimentation marker.*

through a linear 10 to 40% sucrose gradient (35,000 rev/min for 30 min), and a homogeneous peak at about 250*S* was observed. This is similar to the sedimentation rate of purified SV40 virus made in BSC-1 cells (4). If these same virus preparations were treated with deoxyribonuclease all of the ^{3}H-labeled DNA remained acid-precipitable.

These data indicate that 14*S* DNA is enclosed in particles that sediment like SV40 virions and is resistant to deoxyribonuclease. To determine whether these particles contain SV40 capsid proteins, purified virus preparations containing 14*S* ^{3}H-labeled DNA were incubated with rabbit anti-SV40 antiserum for 1 hr at 37 C, followed by sheep anti-rabbit antiserum for 1.5 hr at 37 C. The precipitate that formed was centrifuged out of suspension and the ^{3}H-label was counted as described above.

If the ^{3}H-labeled 14*S* DNA is enclosed in SV40 capsids, one would expect that this DNA should be associated with rabbit anti-SV40 antibody which would then be precipitated by the sheep anti-rabbit antibody. Indeed, 71% of the ^{3}H-label in purified virus preparations could be

precipitated by this indirect precipitation test (total ^3H counts/min, 16,100; ^3H counts/min in precipitate, 11,430).

DNA-DNA hybridization with 14S DNA obtained from virions. It is conceivable that infection of primary AGMK cells, but not CV-1 or BSC-1 cells, resulted in the degradation of cellular DNA (labeled with ^3H-thymidine) to acid-soluble products which were then incorporated into an unusual viral component that sedimented only at 14*S*. To determine the nature of the 14*S* DNA found in virions made in AGMK cells, DNA-DNA hybridization was employed. ^3H-labeled SV40 DNA, AGMK DNA, and 14*S* DNA (each in solution) were hybridized to nitrocellulose filters containing viral, AGMK, or no DNA as described above. As can be observed in Table 1, there is some cross-reaction between viral and cellular DNA (2), but the base sequences of 14*S* DNA behave more like cellular DNA than viral DNA. It is concluded that 14*S* DNA found in purified SV40 virions grown in AGMK cells is cellular DNA.

Density of pseudovirions and size heterogeneity of cellular DNA in pseudovirions. Michel, Hirt, and Weil (13) have shown that a variable fraction of the polyoma pseudovirions have a lighter buoyant density in CsCl then does infectious virus. This is due to the fact that these particles contain less DNA per unit of capsid protein than the true virion. This allows one to enrich the fraction of pseudovirions in a preparation.

The absence of detectable pseudovirions in virus preparations grown in BSC-1 cells allows one to compare the properties of purified SV40 virus with (AGMK-SV40) and without (BSC-1-SV40) pseudovirions present. Purified ^{32}P-labeled SV40 prepared in BSC-1 cells (^{32}P added after infection) was mixed with purified ^3H-labeled SV40 grown in AGMK cells (^3H-labeled prior to infection) and centrifuged to equilibrium in a CsCl gradient. The results of this experiment are presented in Fig. 3. Both the ^3H-labeled virus (AGMK) and ^{32}P-labeled SV40 (BSC-1) banded as a single peak at a density of 1.32 g/ml. The ratio of ^3H to ^{32}P on the dense side of the peak varied between 2.8 and 3.1, whereas this ratio on the lighter side of the peak was between 3.6 and 9.0. These data indicate that the pseudovirions prepared in AGMK cells contain a component(s) that bands on the lighter side of 1.32 g/ml and is not found, or is found to a lesser degree, in virus synthesized in BSC-1 cells. It should be pointed out that pseudovirions band in a CsCl equilibrium gradient throughout the virus peak. They are enriched, relative to true virions, on the lighter density side of the peak.

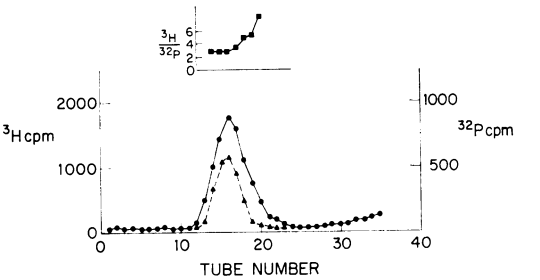

FIG. 3. *CsCl equilibrium gradient of purified SV40 virus made in BSC-1 cells and AGMK cells. SV40 was grown in AGMK cells (^3H-thymidine added before infection) and BSC-1 cells (^{32}P added after infection) and the virus was purified as described. A mixture of SV40-AGMK virus (^3H) and SV40-BSC-1 virus (^{32}P) was centrifuged to equilibrium in CsCl (1.32 g/ml) in an SW39 rotor for 25 hr at 115,000 × g. Symbols: ●, ^3H counts/min; ▲, ^{32}P counts/min; ■, ^3H to ^{32}P ratio.*

TABLE 1. *DNA-DNA hybridization of viral, cellular, and 14S DNA*

Source of DNA (in solution)	Total counts/min	Per cent bound to filter[a]		
		Viral	AGMK	No DNA[b]
SV40, supercoil	5,200	29.1	2.2	0.34
AGMK	86,500	2.0	19.1	0.17
14*S*	18,250	1.8	16.3	0.22

[a] Viral and AGMK DNA, 15 μg/filter.
[b] Backgrounds not subtracted from per cent bound to filters.

To determine whether the pseudovirions band at the lighter density because they contain lower DNA to protein ratios, samples were taken from fractions 16, 18, and 20 of the equilibrium gradient shown in Fig. 3. These were treated with 0.6% SDS, dialyzed as described previously, and sedimented through a 5 to 20% linear sucrose gradient. When needed, ^{32}P-labeled 21*S* viral DNA was added as a sedimentation marker. The results of this experiment are presented in Fig. 4A, B, C. The cellular DNA obtained from pseudovirions banding in fraction 16 (density of 1.32 g/ml) of the equilibrium run (Fig. 3) sedimented at 15*S* with respect to the 21*S* viral DNA marker (Fig. 4A). A small amount of ^3H-label was observed to cosediment with the 21*S* marker. In most experiments, this was not usually observed. Most of the cellular DNA obtained from pseudovirions in fraction 18 (Fig. 3) sedimented at 13*S* (Fig. 4B), whereas the ^3H-labeled cellular DNA in fraction 20 (Fig. 3) sedimented at 11*S* (Fig. 4C). In all cases, the

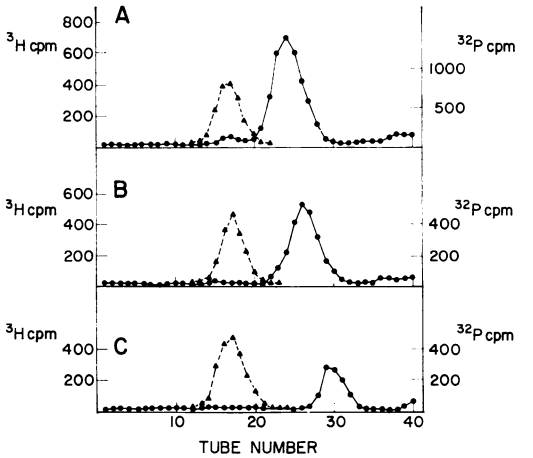

FIG. 4. *Sucrose gradient velocity centrifugation of different density fractions of SV40 virus made in AGMK cells. Purified SV40 virus obtained in Fig. 3 was broken open with SDS and sedimented in an SW50.1 rotor for 3.5 hr at 130,000 × g. A, fraction number 16; B, fraction number 18; C, fraction number 20, all in Fig. 3. Symbols: ●, ^{3}H counts/min; ▲, ^{32}P counts/min added as a sedimentation marker of 21S, viral DNA, when needed.*

^{3}H-labeled peaks obtained by sedimentation through neutral sucrose were quite broad and frequently had a sharper leading edge than trailing edge. These data indicate a distinct size heterogeneity of cellular DNA in SV40 pseudovirions. The buoyant density heterogeneity of these particles is therefore a reflection of the cellular DNA to capsid protein ratios of the pseudovirions themselves.

Alkaline velocity sedimentation of cellular DNA in virus particles. Neutral sucrose sedimentation will determine the comparative sizes of various types of DNA only if all these DNA have the same general type of conformation. To eliminate this complication, purified SV40 virus was prepared in ^{3}H-thymidine-prelabeled AGMK cells as before. The ^{3}H-labeled cellular DNA was obtained from fractions of the CsCl equilibrium gradient as in Fig. 3. Purified virus obtained from these different fractions was dialyzed and broken open in 0.1 M NaOH for 10 min at 37 C. These fractions were then sedimented through a linear 5 to 20% alkaline sucrose gradient. A ^{32}P-labeled 16S (relaxed circular SV40 DNA) marker was added before centrifugation. As shown in Fig. 5A, the ^{3}H-labeled cellular DNA sedimented as a broad peak at 15S. The denatured 16S ^{32}P-marker did not separate into 16S (linear) and 18S (circular) components, because the speeds and time at which this gradient was run did not permit a good separation of these two

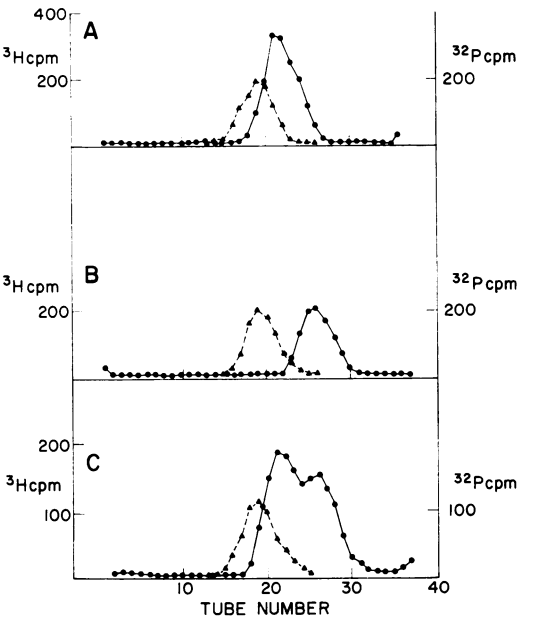

FIG. 5. *Alkaline sucrose gradient velocity centrifugation of different density fractions of SV40. Purified SV40 virus obtained as in Fig. 3 was broken open with 0.1 M NaOH and sedimented through an alkaline sucrose gradient (5 to 20% sucrose, 0.1 M NaOH; 0.9 M NaCl) in an SW50.1 rotor for 3 hr at 130,000 × g. A, fraction 16 (1.32 g/ml of CsCl); B, fraction 20; and C, a mixture of fractions 16 and 20 from a CsCl equilibrium gradient similar to that shown in Fig. 3. Symbols: ●, ^{3}H counts/min; ▲, ^{32}P counts/min added as a 16S, nicked circular SV40 DNA sedimentation marker.*

single strands of DNA. The sedimentation marker was therefore taken as 17S. ^{3}H-labeled cellular DNA obtained from the less dense side of the purified virus peak sedimented through alkaline sucrose with a broad peak at 11S (Fig. 5B). When the samples obtained in Fig. 5A and B were mixed and sedimented through alkaline sucrose gradients (Fig. 5C), a double peak of ^{3}H-label was observed. These data confirm the size heterogeneity of pseudovirion DNA. In addition, these data indicate that the cellular DNA polynucleotide strand(s) labeled before infection and found in the pseudovirion does not contain extensive single-strand breaks.

DISCUSSION

The results presented in this paper demonstrate that cellular DNA can be found in purified SV40 virions (pseudovirions) grown in primary AGMK cells. Two different kinds of experiments lead to this conclusion. (i) Purified SV40 virions obtained from primary AGMK cells labeled before

infection with ^{3}H-thymidine contain ^{3}H-labeled DNA. This DNA is most likely in the virion since it is resistant to deoxyribonuclease, sediments at 250*S*, and can be precipitated with antiserum to purified SV40 virions. When the virion is broken open, the ^{3}H-label sediments at 14*S*. Little or none of the ^{3}H is observed to sediment at 21*S*. (ii) The 14*S* DNA obtained from purified virions behaves like cellular DNA in a DNA-DNA hybridization test. These results are identical to those obtained with polyoma virus (13, 18).

The 14*S* cellular DNA can be observed in pseudovirions made in AGMK cells even if the ^{3}H-thymidine is added after infection. In this case, both viral DNA and cellular DNA (14*S*) are labeled. Thus, pseudovirions contain some cellular DNA made before infection and some cellular DNA synthesized after infection. The quantity of pseudovirions found in SV40 virus preparations appears to be quite variable. When primary AGMK cells are labeled with ^{3}H-thymidine before infection, the virus preparations obtained from these cells contain between 0.03 and 0.3% of the total cellular ^{3}H-labeled DNA. Some of this DNA must be redundant or have regions of similar base sequences (5), since the DNA concentration and the time of incubation of the DNA-DNA hybridization test would not permit hybridization of the unique fraction of monkey cell DNA base sequences (5). It is not clear, however, whether the cellular DNA in pseudovirions is a general representation of all cellular DNA or a rather specific fraction of the genome.

Little or no cellular DNA was found in SV40 virions grown in CV-1 cells or BSC-1 cells The results presented here, however, do not eliminate a small amount of pseudovirion production in these cells. In this paper, a direct comparison of the quantity of cellular DNA found in SV40 virions made in AGMK cells with that observed in BSC-1 or CV-1 cells is not quite fair. This is because the SV40 large-plaque virus was used in AGMK cells, whereas the wild-type virus was employed for growth in BSC-1 and CV-1 cells. The large-plaque virus [which grows poorly in BSC-1 or CV-1 cells (14, 15)] produces two to four times greater quantities of SV40 virus in AGMK cells than does the wild-type virus. The wild-type virus preparations do contain pseudovirions when grown in AGMK cells but the amounts produced are correspondingly lowered.

It is interesting to note that polyoma pseudovirions were detected by growing polyoma virus in a primary mouse cell line (13). Analogously, SV40 pseudovirions are obtained only in primary AGMK cells. The reason that SV40 does not produce pseudovirions in BSC-1 cells is that the virus does not cause an induction of host-cell DNA synthesis nor excision of cellular DNA from the genome (Ritzi and Levine, *in preparation*). In polyoma-infected cells, induction of cellular DNA synthesis is required for excision of host-cell DNA (3). In CV-1 cells, induction of host-cell DNA synthesis is normal, but excision of cellular DNA is much reduced or nonexistent (Ritzi and Levine, *in preparation*).

The SV40 pseudovirions exhibit a spectrum of densities in CsCl equilibrium gradients. This is due to the size heterogeneity of the cellular DNA enclosed in SV40 coat proteins. The 14*S* DNA obtained from these pseudovirions has the same buoyant density in CsCl as does viral and cellular DNA (1.702 g/ml; 6). Pseudovirions in an SV40 virus preparation are enriched on the light density side of the virus peak in a CsCl equilibrium gradient (Fig. 3). If virus preparations are obtained from infected AGMK cells labeled with ^{3}H-thymidine after infection, the PFU to ^{3}H ratio is lower on the lighter density side of the virus peak in a CsCl equilibrium gradient than on the heavier side (Fig. 1). A similar relationship between PFU and optical density has been reported when purified SV40 virions grown in primary green monkey kidney cultures are banded to equilibrium in CsCl (20). These data are consistent with the expectation that pseudovirions are not infectious. The possibility that SV40 pseudovirions transduce cellular genetic markers remains to be explored.

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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. Aloni, Y., E. Winocour, L. Sachs, and J. Torten. 1969. Hybridization between SV40 DNA and cellular DNA's. J. Mol. Biol. 44:333–345.
3. Ben-Porat, T., C. Coto, and A. S. Kaplan. 1966. Unstable DNA synthesized by polyoma virus-infected cells. Virology 30:74–81.
4. Black, P. H., E. M. Crawford, and L. V. Crawford. 1964. The purification of simian virus 40. Virology 24:381–387.
5. Britten, R. J., and D. E. Kohne. 1968. Repeated sequences in DNA. Science 161:529–540.
6. Crawford, L. V., and P. H. Black. 1964. The nucleic acid of simian virus 40. Virology 24:388–392.
7. Denhardt, D. 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. 23:641–646.
8. Dulbecco, R., L. H. Hartwell, and M. Vogt. 1965. Induction of cellular DNA synthesis by polyoma virus. Proc. Nat. Acad. Sci. U.S.A. 53:403–410.
9. 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.

10. Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
11. Kamano, T., and R. L. Sinsheimer. 1968. Preparation and purification of ϕX-RF component 1. Biochim. Biophys. Acta **155**:295–298.
12. Levine, A. J., and H. S. Ginsberg. 1967. Mechanism by which fiber antigen inhibits multiplication of type 5 adenovirus. J. Virol. **1**:747–757.
13. 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.
14. 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.
15. 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.
16. Weil, R., M. Michel, and G. Ruschman. 1965. Induction of cellular DNA synthesis by polyoma virus. Proc. Nat. Acad. Sci. U.S.A. **53**:1468–1475.
17. Winocour, E. 1967. Studies on the basis of the observed homology between DNA from polyoma virus and DNA from normal mouse cells, p. 577–591. *In* J. S. Colter and W. Paranchych (ed.), The molecular biology of viruses. Academic Press Inc., New York.
18. Winocour, E. 1969. Some aspects of the interaction between polyoma virus and cell DNA. Advan. Virus Res. **14**:153–200.
19. Winocour, E., A. M. Kaye, and V. Stollar. 1965. Synthesis and transmethylation of DNA in polyoma infected cells. Virology **27**:156–169.
20. Uchida, S., K. Yoshiike, S. Watanabe, and A. Finuno. 1968. Antigen-forming defective viruses of simian SV40. Virology **34**:1.