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Early Events in the Infection of Permissive Cells with Simian Virus 40: Adsorption, Penetration, and Uncoating

GIUSEPPE BARBANTI-BRODANO,[1] PETER SWETLY,[2] AND HILARY KOPROWSKI
The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

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The early events in the interaction of simian virus 40 (SV40) with permissive cells were investigated. Evidence is presented that 30 min after infection intact virions penetrate the nuclei of infected cells. The uncoating of the virus is carried out in the nuclei with a complete dissociation of the viral genome from the protein coat. Opening of the circular parental deoxyribonucleic acid (DNA), i.e., conversion of component I to component II of SV40 DNA, takes place after uncoating, followed by the appearance of a new component sedimenting faster than component I at alkaline *p*H.

In a previous report (18), we showed the resistance to superinfection with simian virus 40 (SV-40) and SV40 deoxyribonucleic acid (DNA) of SV40-transformed nonpermissive cells. In addition, we found that SV40-transformed permissive cells are resistant to superinfection with SV40, but susceptible to SV40 DNA. Hence, the infection of SV40-transformed permissive cells with intact virus is probably blocked at an early stage, before the replication of viral DNA occurs.

A comparison of the early events of infection in SV40-transformed permissive cells with the same phase of the infectious cycle in nontransformed permissive cells may elucidate the nature of this block.

The early events of cell-virus interaction in nontransformed permissive cells have not been extensively investigated. The present report describes the adsorption, penetration, and uncoating of SV40 in such cells.

MATERIALS AND METHODS

Cells. Primary and secondary African green monkey kidney (AGMK) cells and CV-1 cells, a continuous line derived from AGMK cells (8), were grown in Eagle's basal medium supplemented with 10% fetal bovine serum (FBS). They were maintained, after infection with SV40, in the same medium supplemented with 2% FBS.

Virus. The Rh 911 strain of SV40 was grown in CV-1 cells as described previously (18).

Labeling of the virus. Monolayer cultures of CV-1 cells were infected at an input multiplicity of 50 plaque-forming units (PFU) per cell. To prepare virus isotopically labeled in the DNA, the medium was removed 15 hr after infection and replaced with fresh medium containing 5 μc of ^{3}H-thymidine (specific activity, 17 c/mmole; New England Nuclear Corp., Boston, Mass.) per ml. To prepare virus isotopically labeled in the proteins, the medium was removed 30 hr after infection and replaced with fresh medium containing one-third of the normal amount of cold leucine (17 μg/ml) and 5 μc of ^{3}H-leucine (specific activity, 40 c/mmole; Schwarz BioResearch Inc., Orangeburg, N.Y.) per ml. The infected cultures were harvested 72 to 96 hr after infection, when the cytopathic effect involved 75 to 100% of the cells. The virus was purified as described previously (18), except that treatment with trypsin was omitted because it was found to decrease the infectivity.

When the purified virus preparations were stained with potassium phosphotungstate and examined with an electron microscope, they were seen to contain 3 to 5% coreless virions. After equilibrium centrifugation in a CsCl solution (106,000 $\times$ *g* for 20 hr), 93% of the radioactivity contained in the purified virus preparations was recovered in a single band located at a density range of 1.30 to 1.38 g/cc. The density at the peak of the band was 1.34 g/cc. The ^{3}H-thymidine-labeled and purified virus contained 2.5×10^6 counts per min per ml and 6.5×10^{10} PFU per ml. The ^{3}H-leucine-labeled virus contained 1.6×10^6 counts per min per ml and 3.4×10^{10} PFU per ml.

Isolation of ^{3}H-thymidine-labeled SV40 DNA. SV40 DNA was extracted from a ^{3}H-thymidine-labeled and purified virus preparation as described previously (18).

Infection of cells with labeled and purified virus. Confluent monolayer cultures of AGMK cells were infected with ^{3}H-thymidine- or ^{3}H-leucine-labeled SV40. The virus was adsorbed for 2 hr at 37 C. The

[1] Fellow of The Wistar Institute from the Institute of Microbiology, University of Bologna, Bologna, Italy.

[2] Fellow of The Wistar Institute from the Institut für Biochemie, University of Vienna, Vienna, Austria.

inoculum was removed and the cell monolayer was washed three times with phosphate-buffered saline (PBS). After the addition of maintenance medium, the cells were incubated at 37 C. (The term "after infection" used in the description of all experiments refers *not* to the time interval after the 2-hr adsorption but to the time interval after the addition of virus.)

Disruption of cells and fractionation of cellular components. Three techniques were used for the disruption of cells and the fractionation of cellular components.

Method A. At various intervals after infection, cell monolayers were washed three times with PBS. The cells were dispersed with trypsin and sedimented by centrifugation at 1,000 × *g* for 4 min. The pelleted cells were suspended in PBS, sedimented again, resuspended in 1 ml of a 0.25 M sucrose solution, and homogenized in a glass tissue grinder (Ten-Broeck type; Bellco Glass, Inc., Vineland, N.J.) at 0 C until more than 90% of the cells were broken. The cell homogenate was fractionated by differential centrifugation at 4 C into three fractions: a nuclear fraction (sediment obtained after centrifugation at 600 × *g* for 5 min), a large-granule fraction containing mitochondria and lysosomes (sediment obtained after centrifugation of the 600 × *g* supernatant fluid at 10,000 × *g* for 30 min), and a cytoplasmic supernatant (supernatant fluid obtained after centrifugation at 10,000 × *g* for 30 min). This procedure was similar to that described by Silverstein and Dales (17).

Method B. Cells were harvested as described in method A and sedimented at 1,000 × *g* for 4 min at 4 C. The pelleted cells were washed once with NTM buffer [0.14 M NaCl, 0.01 M tris(hydroxymethyl)-aminomethane (Tris)-chloride, 0.0015 M $MgCl_2$, *p*H 7.4] and sedimented again. The cells were disrupted by resuspending them in 2 ml of 0.5% Nonidet P-40 (NP-40) nonionic detergent (Shell Chemical Co.) in NTM buffer (1, 13). After incubation at 0 C for 10 min with occasional shaking, the suspension was centrifuged at 1,600 × *g* for 2 min at 4 C. The cytoplasmic supernatant fluid was decanted, and the sedimented nuclei were washed once in PBS. Nuclei from 65 to 80% of the cells contained in the original cell cultures were recovered by this method. No contamination with cytoplasmic components could be detected when the final nuclear preparation was examined by phase-contrast microscopy.

Method C. Nuclei were isolated according to a procedure described by Penman (15).

Isopycnic centrifugation in CsCl solution of SV40 recovered from nuclei of infected cells. Nuclei isolated from infected AGMK cells were suspended in 0.9 ml of 0.5% NP-40 in NTM buffer. A 0.1-ml amount of 10% sodium deoxycholate (DOC) in distilled water was added, and the mixture was incubated for 10 min at 37 C. The lysed nuclei were mixed with 4 ml of 45.5% (w/v) CsCl solution in 0.02 M Tris-chloride, *p*H 7.4 (average density, 1.33 g/cc), and centrifuged for 20 hr at 4 C at 106,000 × *g* in the SW 39 rotor of a Spinco centrifuge. Three-drop fractions were then collected from the bottom of the tubes and assayed for radioactivity. The density of the fractions was calculated from the refractive index (7), determined in an Abbé refractometer.

Rate zonal centrifugation in alkaline sucrose gradients. Nuclei isolated from infected AGMK cells were suspended in 0.3 ml of 0.5% DOC in distilled water and layered on top of 5 to 20% (w/v) linear sucrose density gradients (5 ml) in 0.8 M NaCl and 0.2 M NaOH (*p*H 12.4). The contents of the tubes were centrifuged at 106,000 × *g* for 90 min at 4 C in the SW 39 rotor of a Spinco centrifuge. Three-drop fractions were collected from the bottom of the tubes and the radioactivity was assayed.

Treatment with deoxyribonuclease and phosphodiesterase. Cytoplasmic and nuclear fractions of AGMK cells infected with ^{3}H-thymidine-labeled SV40 were treated with 200 μg of pancreatic deoxyribonuclease per ml (deoxyribonuclease I, Sigma Chemical Co., St. Louis, Mo.) and 0.1 unit of phosphodiesterase per ml (Sigma Chemical Co.) in the presence of 0.02 M $MgSO_4$ for 30 min at 37 C. The nuclease-resistant material was then precipitated with trichloroacetic acid, and the radioactivity was determined. The uncoating of viral DNA was evaluated according to Joklik (9) on the basis of the amount of nuclease-sensitive acid-precipitable radioactivity present in the cells after infection with ^{3}H-thymidine-labeled SV40.

The presence of NP-40 and DOC in the cellular fractions did not inhibit the action of nucleases, as has also been reported for adenovirus type 5 (10), and did not affect the buoyant density of the viral particles.

Determination of radioactivity. Total radioactivity was determined by addition of 20− or 40−μliter samples to glass-fiber filters (934 AH; diameter, 2.4 cm; H. Reeve Angel & Co., Inc., Clifton, N.J.). The filters were dried, and the radioactivity was counted in 10 ml of Liquifluor (New England Nuclear Corp.) diluted in toluene. The radioactivity of the acid-precipitable material was determined by the addition of 30 μg of carrier yeast ribonucleic acid per ml and trichloroacetic acid to a concentration of 5%. The samples were kept at 0 C for 1 hr. The precipitate was collected by filtration through glass-fiber filters and dried. The radioactivity was measured by counting for 10 min in a Packard liquid scintillation spectrometer.

RESULTS

Kinetics of adsorption of SV40 onto AGMK cells. Within 15 min, 30% of the input radioactivity of the ^{3}H-thymidine-labeled virus became cell associated. The rate of adsorption then reached a plateau, 50% of the total input radioactivity being adsorbed 2 hr after infection (Fig. 1).

To determine whether SV40 was bound firmly to the receptors of the cell membrane, AGMK cells infected with ^{3}H-thymidine-labeled virus were harvested 10 min after infection and disrupted; the cell homogenate was then centrifuged in a neutral CsCl density gradient (Fig. 2). The bulk of the cell-associated radioactivity banded at the density of 1.27 g/cc, indicating binding of the virus to lipoprotein-containing cell components, and only 10% of the radioactivity was recovered

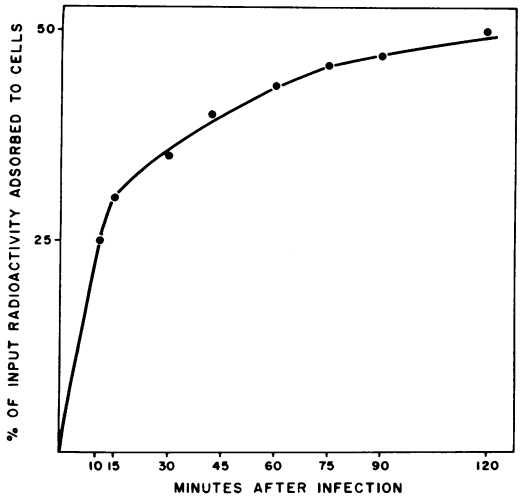

FIG. 1. *Kinetics of adsorption of SV40 onto AGMK cells. Confluent monolayers containing 3 × 10⁶ AGMK cells were infected with ³H-thymidine-labeled SV40 at an input multiplicity of 100 PFU/cell (input radioactivity, 1.5 × 10⁴ counts per min per culture). The infected cultures were incubated at 37 C. At intervals after infection, the inoculum was removed; the cells were washed three times with PBS and harvested into a final volume of 2 ml. The acid-precipitable radioactivity contained in the cell suspension was then determined.*

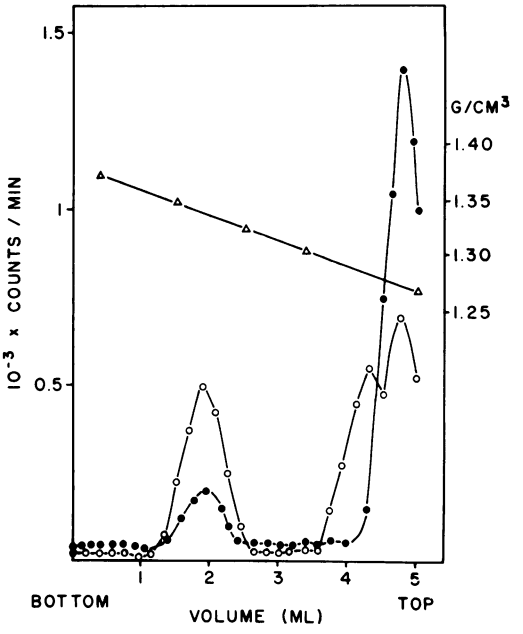

FIG. 2. *Association of SV40 with cell membrane components. Confluent monolayers containing 6 × 10⁶ AGMK cells were infected with ³H-thymidine-labeled SV40 at an input multiplicity of 100 PFU/cell (input radioactivity, 3 × 10⁴ counts per min per culture). At 10 min after infection, the cells were washed three times with PBS and scraped into 5 ml of PBS. The cell suspension was then centrifuged at 1,000 × g for 5 min at 4 C. The sedimented cells were resuspended in 1 ml of RSB buffer of Penman (15) and broken with 25 strokes in a tight-fitting Dounce homogenizer. One-half of the cell homogenate was layered on top of a preformed linear CsCl density gradient (average density, 1.33 g/cc) and centrifuged at 106,000 × g for 5 hr at 4 C in the SW 39 rotor of a Spinco L centrifuge. The other half of the cell homogenate was incubated with 0.5% DOC at 37 C for 20 min before layering on top of the gradient. Fractions of 3 drops were collected from the bottom of the gradients, and the radioactivity was determined. Symbols: △, density at 25 C; ●, radioactivity of ³H-thymidine; ○, radioactivity of ³H-thymidine after treatment with DOC.*

in the form of free virus banding at a density of 1.34 g/cc. After treatment of the cell homogenate with DOC, 33% of the radioactivity recovered before treatment at a density of 1.27 g/cc was shifted to the density of free SV40 particles.

Distribution of SV40 and its components in infected cells. The distribution of SV40 in the infected cells was determined in the following way. Cells infected with ³H-thymidine- or ³H-leucine-labeled virus were harvested at intervals after infection, disrupted, and fractionated. The radioactivity contained in the acid-precipitable cellular fractions was then determined. The results shown in Table 1 indicate that at 30 min after infection most of the cytoplasmic radioactivity was associated with large granules, although an appreciable quantity of label was also found at that time in the nuclei. The relative amount of ³H-leucine radioactivity recovered from the nuclei was higher than that of ³H-thymidine. This observation was confirmed in repeated experiments.

At 60 min after infection, the radioactivity associated with the nuclei increased significantly, whereas that associated with the large-granule fraction decreased correspondingly. The transfer of cytoplasmic radioactivity from the large-granule fraction to the nuclei continued for up to 120 min after infection. Less than 10% of the total intracellular radioactivity was recovered in the cytoplasmic supernatant fluid between 30 and 120 min after infection.

Kinetics of uncoating of SV40 in the cytoplasm and nuclei of infected cells. The distribution of the ³H-leucine- and ³H-thymidine-labeled viral components between the cytoplasm and the nuclei of infected cells was determined for the period from 0.5 to 24 hr after infection. At the same time, so that the uncoating of SV40 in the cytoplasm and in the nuclei might be investigated, the sensitivity

TABLE 1. *Distribution of SV40 in different fractions of infected cells*[a]

Fraction	Percentage of cell-associated radioactivity at various intervals after infection					
	^{3}H-thymidine			^{3}H-leucine		
	30 min	60 min	120 min	30 min	60 min	120 min
Nuclei	10	32	50	36	58	66
Large granules	84	64	43	59	36	26
10,000 × *g* supernatant fluid	6	4	7	5	6	6

[a] Monolayer cultures containing 6×10^6 AGMK cells were infected with ^{3}H-thymidine- or ^{3}H-leucine-labeled SV40 at an input multiplicity of 2 PFU/cell (input radioactivity, 6×10^2 counts per min per culture). Cells were harvested at intervals after infection, homogenized, and fractionated by method A described in Materials and Methods. The acid-precipitable radioactivity contained in the fractions was then determined.

of the viral DNA to the degrading action of nucleases was determined (Fig. 3).

The time dependence of the distribution of the ^{3}H-leucine and ^{3}H-thymidine viral radioactivity in the nuclei and in the cytoplasm followed a similar pattern. At 2.5 hr after infection, approximately 60% of both the ^{3}H-thymidine and ^{3}H-leucine cell-associated radioactivity was found in the nuclei. Between 2.5 and 4 hr after infection, the release of both radioactive precursors from the nuclei was accompanied by a complementary increase in the radioactivity recovered in the cytoplasmic fraction. Later, a gradual decrease in acid-precipitable radioactivity of both the nuclear and cytoplasmic fractions was observed. Between 3 and 5% of the cell-associated ^{3}H-thymidine or ^{3}H-leucine acid-precipitable radioactivities were recovered in the medium at any time between 2 and 24 hr after infection. About one-third of the acid-precipitable ^{3}H-thymidine radioactivity contained in the medium was degraded by the action of nucleases.

SV40 DNA contained in the cytoplasmic fraction (Fig. 3a) displayed sensitivity to nucleases as early as 0.5 hr after infection. This sensitivity reached a maximum at 4 hr, when 50% of the acid-precipitable ^{3}H-thymidine radioactivity contained in the cytoplasm was digested by nucleases. In contrast, the intranuclear viral DNA was almost completely refractory to the action of nucleases up to 1.5 hr after infection (Fig. 3b), whereas at 2.5 hr and later about 60% of the acid-precipitable ^{3}H-thymidine radioactivity became sensitive to the action of the enzymes.

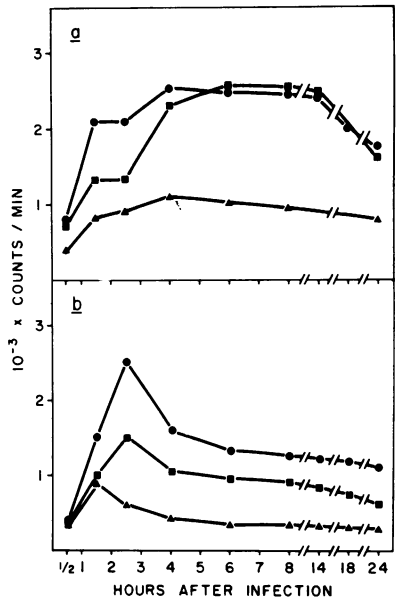

FIG. 3. *Distribution of SV40 proteins and SV40 DNA between the cytoplasm and the nuclei of infected cells and sensitivity of the viral DNA to nucleases. Monolayer cultures containing 6×10^6 AGMK cells were infected with ^{3}H-thymidine- or ^{3}H-leucine-labeled SV40 at an input multiplicity of 100 PFU/cell (input radioactivity, 3×10^4 counts per min per culture). Cells were harvested at intervals after infection, and the nuclei were separated from the cytoplasm by method B, described in Materials and Methods. The acid-precipitable radioactivity contained in the cytoplasmic (a) and in the nuclear (b) fractions was determined. In cells infected with ^{3}H-thymidine-labeled virus, the acid-precipitable radioactivity of the fractions was determined also after treatment with deoxyribonuclease and phosphodiesterase. Each point represents mean values from five independent experiments. Symbols: ●, ^{3}H-leucine radioactivity; ■, ^{3}H-thymidine radioactivity; ▲, ^{3}H-thymidine radioactivity refractory to the action of deoxyribonuclease and phosphodiesterase.*

Isolation of SV40 particles from nuclei of infected cells. The experiments described above have shown that both viral proteins and viral DNA can be recovered from the nuclei of infected cells soon after infection. To determine whether intact virions penetrate the nuclei, AGMK cells were infected with ^{3}H-thymidine- or ^{3}H-leucine-labeled SV40 and were harvested at intervals after infection; their nuclei were then analyzed for the presence of SV40 particles (Fig. 4 and 5). Between 0.5 and 2.5 hr after infection, both ^{3}H-thymidine and ^{3}H-leucine radioactivity recovered from the disrupted nuclei banded after equilibrium density gradient centrifugation at a density of 1.34 g/cc. Later, the density of the ^{3}H-thymidine-labeled viral components recovered

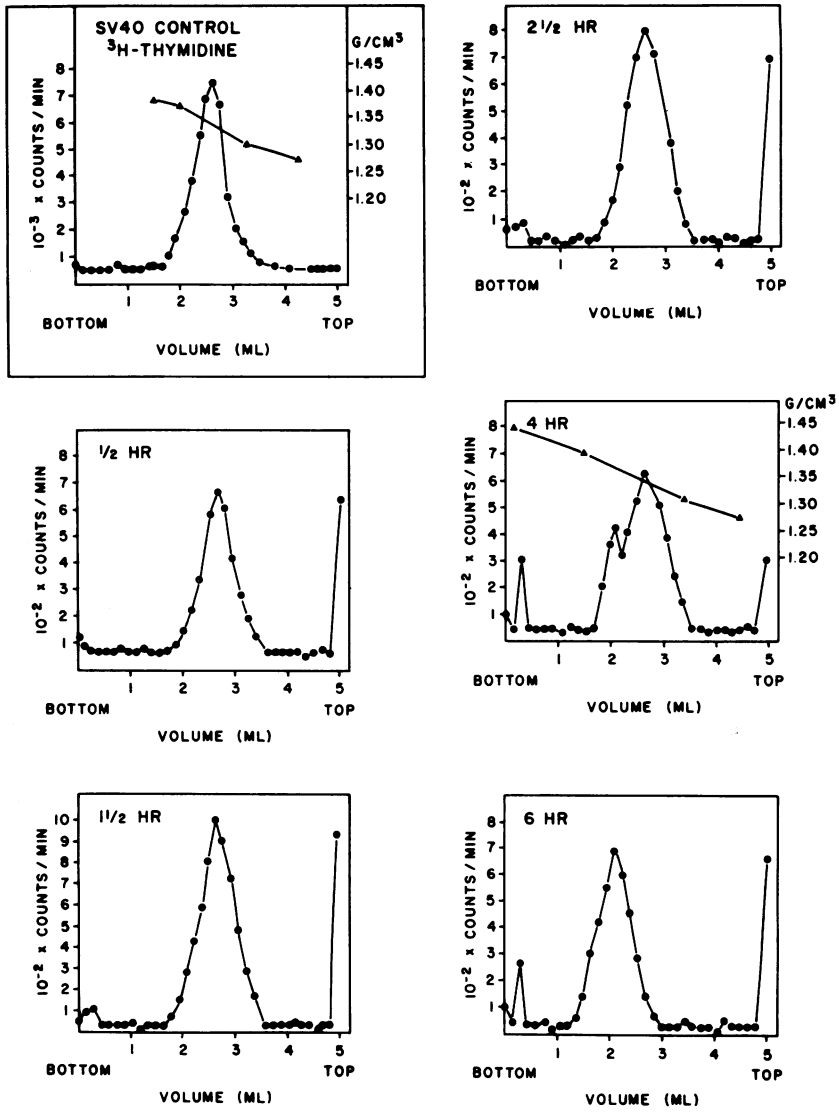

FIG. 4. *Isolation of parental SV40 from nuclei of infected cells. Confluent monolayer cultures containing 6 × 10⁶ AGMK cells were infected with ³H-thymidine-labeled SV40 at an input multiplicity of 100 PFU/cell (input radioactivity, 3 × 10⁴ counts per min per culture). At intervals after infection cells were harvested, and the nuclei were isolated by method B, described in Materials and Methods. Nuclei were disrupted and analyzed by equilibrium centrifugation in CsCl density gradients. In parallel, purified and labeled SV40 preparations used for the infection of cells were analyzed. Symbols: ▲, density at 25 C; ●, total radioactivity.*

from the nuclei shifted gradually to a density of 1.37 g/cc. A minor component banding at a density of 1.43 g/cc could also be detected at 4 hr after infection (Fig. 4). Between 4 and 6 hr after infection, the density of the ³H-leucine-labeled material recovered from the nuclei shifted gradually from 1.34 to 1.30 g/cc (Fig. 5). About 20% of both the ³H-thymidine and ³H-leucine radioactivity contained in the nuclei was found at the top of the gradients.

The results of these experiments indicate that from 0.5 to 2.5 hr after infection intact virions can be recovered from the nuclei of infected AGMK cells. Between 2.5 and 4 hr after infection, intranuclear virions started to be uncoated. The protein coat of the virus was completely removed from the viral genome, and the parental DNA became associated with either cellular or newly synthesized viral proteins.

Fate of parental SV40 DNA in the nuclei of

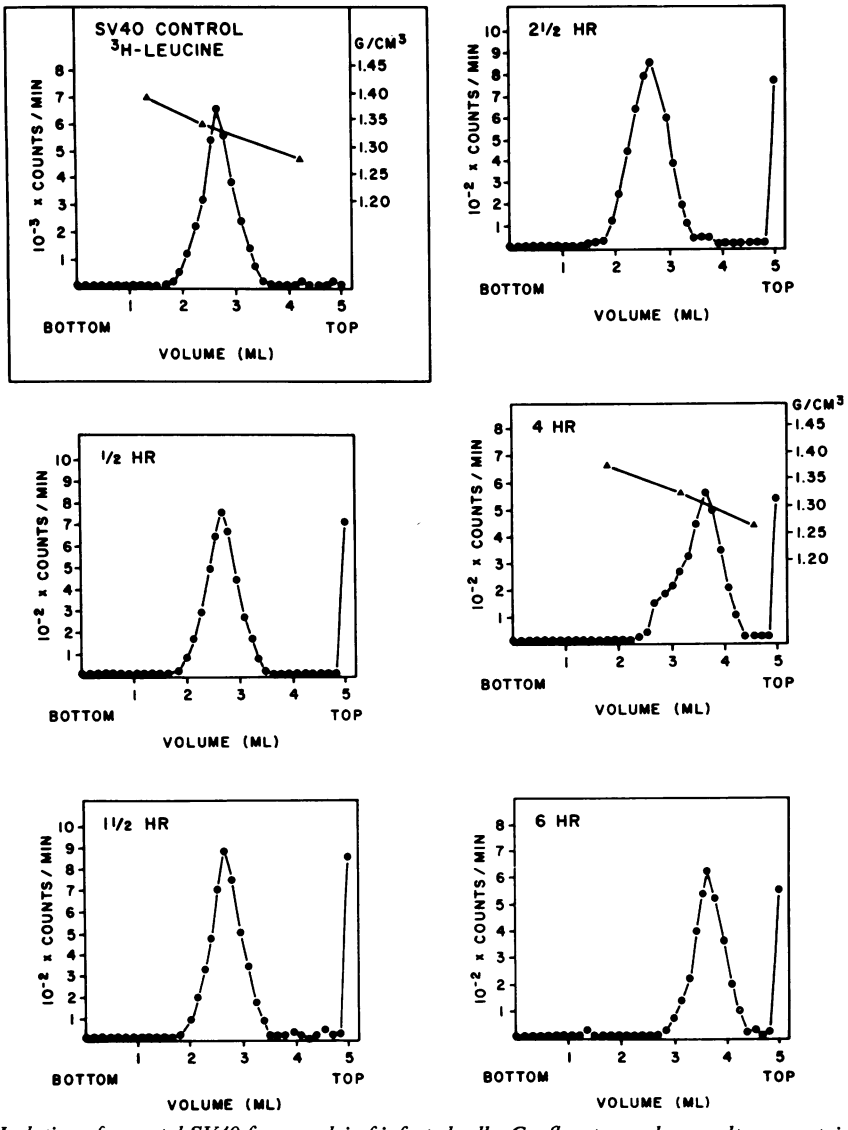

FIG. 5. *Isolation of parental SV40 from nuclei of infected cells. Confluent monolayer cultures containing 6 × 10⁶ AGMK cells were infected with ³H-leucine-labeled SV40 at an input multiplicity of 100 PFU/cell (input radioactivity, 3 × 10⁴ counts per min per culture). Experimental conditions and symbols are as described in the legend to Fig. 4.*

infected cells. The state of intranuclear SV40 DNA was investigated by rate zonal centrifugation in alkaline sucrose gradients of nuclei isolated from cells harvested at various intervals after infection.

The sedimentation properties of the intranuclear viral DNA were compared with those of ³H-thymidine-labeled SV40 DNA extracted from purified virus preparations (Fig. 6). After denaturation in alkaline solution, component I of SV40 DNA (2) has a sedimentation coefficient of 53*S* [the same as that described for the DNA of the related polyoma virus (19)], whereas component II of SV40 DNA (2) is usually separated in two single-stranded DNA fractions sedimenting at 18*S* and 16*S* (19). However, under the conditions of centrifugation used in these experiments, the single-stranded circular and linear forms of component II were not resolved.

Viral DNA extracted from the purified virions contained about 90% of component I and 10% of component II, whereas intranuclear viral DNA isolated from the cells as early as 2.5 hr after infection contained 34% of component I, 26% of component II, and 25% of an unidentified com-

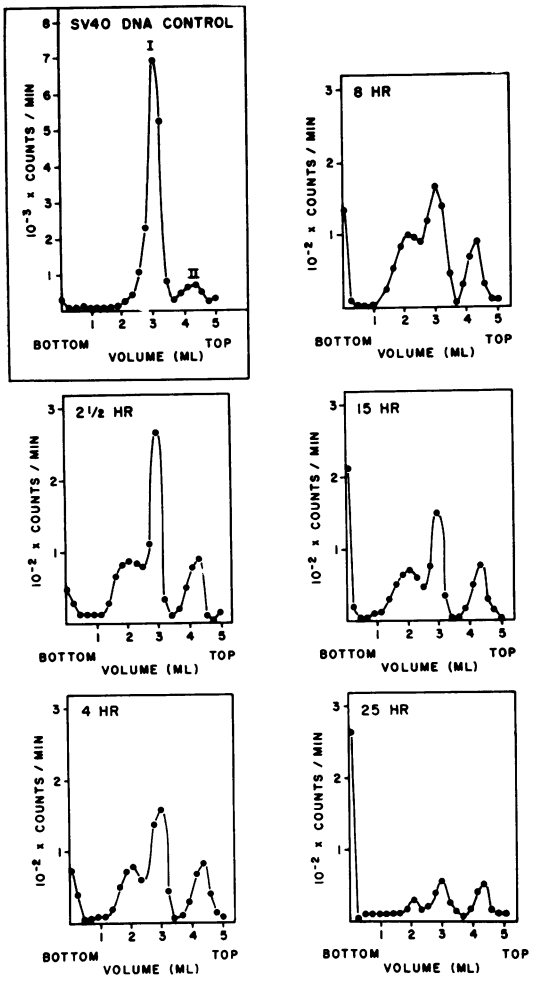

FIG. 6. *Fate of parental SV40 DNA in the nuclei of infected cells. Monolayer cultures containing 6 × 10^6 AGMK cells were infected with ^{3}H-thymidine-labeled SV40 at an input multiplicity of 50 PFU/cell (input radioactivity, 1.5 × 10^4 counts per min per culture). Nuclei were isolated from cells at intervals after infection by method B, described in Materials and Methods. The nuclear suspension was layered on top of an alkaline sucrose density gradient, centrifuged, and analyzed as described in Materials and Methods. Sedimentation coefficients were determined as described by Martin and Ames (12).*

ponent sedimenting at 76S. The band corresponding to the 76S component was always broader than those corresponding to component I and component II, indicating a high degree of heterogeneity of the 76S component. Up to 15 hr after infection, the relative proportion of component II and of the 76S component, as well as of the DNA sedimenting to the bottom of the gradient, increased while that of component I decreased. At 25 hr after infection, a decrease in the total amount of component I, component II, and the 76S component was observed, and the amount of DNA sedimenting to the bottom of the gradient was markedly increased.

DISCUSSION

As previously reported for different SV40 mutants (14), the adsorption of SV40 to permissive cells is a rapid process, and 50% of the input virus is adsorbed within 2 hr after infection. The association of viral radioactivity at 10 min after infection with lipoprotein-containing cell components suggests that the virus adsorption is mediated by a combination of the viral particles with cellular receptors. The binding of the virus to the cell membrane is strong, because the virus-receptor complex is not dissociated in a CsCl solution and viral particles are only partially released after treatment with DOC. It is possible, however, that intracellular components are also bound to the virus as early as 10 min after infection, because intracytoplasmic virions can be detected in this phase of infection by electron microscopic techniques (6).

The association of most of the intracellular virus with the large cytoplasmic granules soon after infection indicates that, after penetration, viral particles are not in a free state in the cytoplasm of the infected cells, but are bound to discrete cytoplasmic structures, probably pinocytotic vacuoles formed after the engulfment of the virions into the cells. Similar electron microscopic observations have been described for most of the animal viruses (3), as well as for SV40 (6).

The simultaneous shift of viral proteins and viral DNA from the cytoplasm to the nuclei, observed between 0.5 and 2.5 hr after infection, indicates that the whole virus penetrates the nucleus. Intact virions were indeed recovered from the nuclei. The possibility of cytoplasmic contamination of the nuclear fraction, and of virus particles being attached to the nuclear membranes rather than penetrating the nucleus, was excluded by the use of the Penman method for isolation of the nuclei. When this procedure, which strips away the "contaminating" cytoplasm and the outer membrane from the nucleus (5), was followed, the amount of SV40 virions recovered from the nuclei was similar to that obtained by other methods. These findings were confirmed by electron microscopic observations of intact virus particles present in the nucleoplasm of AGMK cells as early as 1 hr after infection with SV40 (6).

The sensitivity of the intranuclear viral DNA to nucleases indicates that the uncoating of the virus is carried out within the nucleus. In fact, the intranuclear uncoating seems to be an en-

dogenous process starting from intact viral particles, since the virions in the nuclei of infected AGMK cells show the same size and morphology (6) and have the same buoyant density in CsCl as those contained in purified SV40 preparations.

At present, it is difficult to decide whether cytoplasm plays any role in the uncoating of SV40. As we have observed, the cytoplasmic viral DNA is sensitive to nucleases as early as 0.5 hr after infection. The viral coat proteins released are rapidly transferred to the nucleus, as indicated by the high ^{3}H-leucine to ^{3}H-thymidine ratio observed in the nuclei early after infection. These findings may represent a true uncoating, resulting in the release of functional viral DNA, or a degradation of viral particles, because of their possible association with lysosomes (large-granule fraction) soon after infection.

It is reasonable to exclude the possibility that partially uncoated virus reaches the nucleus after being processed in the cytoplasm, because no changes in the buoyant density of the virions are detected in the nuclei until 4 hr postinfection. However, it cannot be ruled out that traces of naked viral DNA penetrate the nuclei, as may be suggested by the changes observed in the parental viral DNA at 2.5 hr after infection. At this time, the intranuclear uncoating has not yet started, but a small amount of ^{3}H-thymidine-labeled material sedimenting at the bottom of the gradient may represent free viral DNA. On the other hand, the difficulty in exactly reproducing the time sequence of the early events, probably due to the different batches of AGMK cells used, may explain the variations observed in different experiments when the intranuclear uncoating of the virus or the fate of the parental viral DNA was followed. These results suggest that the products of uncoating in the nuclei originate from the intranuclear viral particles, even though small amounts of viral proteins and DNA can be transferred separately from the cytoplasm to the nucleus. The last steps of uncoating of adenovirus type 5 are also performed in the nucleus (11).

Although small amounts of viral DNA (1% of the total intracellular radioactivity) are released from the cells into the medium, the cell surface does not seem to be involved in the uncoating phenomenon [in contrast to what has been reported for enteroviruses (4, 16; V. F. Chan and F. L. Black, Fed. Proc. **28**:433, 1969)], since the production of infectious SV40 was not decreased by treatment of the cells with deoxyribonuclease during the period of virus adsorption (*unpublished observations*).

Thus, it seems most likely that SV40 replication is initiated by the virus which reaches the nucleus. After uncoating, the parental viral DNA seems to form a complex with cellular or newly synthesized viral proteins. With the sequence of changes of the parental SV40 DNA in the nuclei during and after uncoating, a gradual conversion of component I to component II of SV40 DNA occurs. This represents breaks in the circular structure of the viral genome (19), probably caused by a nickase in order to allow replication of the viral DNA.

An increase, up to 25 hr after infection, in the radioactive material recovered at the bottom of the gradient most likely indicates a progressive degradation of viral DNA with subsequent reutilization of the labeled precursor for cellular DNA synthesis. Further investigation could elucidate whether the 76*S* component appearing 2.5 hr after infection is of viral origin and whether it is related to the replicative form of SV40 DNA.

Finally, the possibility must be considered that the parental viral coat proteins present in the nuclei, where the viral genome expresses its genetic information, may play some role in the regulation of the transcription and replication of viral DNA.

ACKNOWLEDGMENTS

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