

# Nucleoprotein Complexes in Simian Virus 40-Infected Cells

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When African green monkey kidney cells (BSC-1) were infected with simian virus 40 (SV40) and extracted with 0.25% Triton X-100 after exposure to  $^3\text{H}$ -thymidine, the  $^3\text{H}$ -SV40 deoxyribonucleic acid (DNA) was present in a form which had a sedimentation coefficient in sucrose gradients of 44S. The change from the sedimentation coefficient of purified SV40 DNA (21S) was shown to result from the association of the SV40 DNA in the Triton extracts with protein by means of sensitivity to Pronase digestion and labeling with  $^{14}\text{C}$ -amino acids. Short-term labeling experiments with  $^3\text{H}$ -thymidine demonstrated that SV40 DNA molecules in the course of replication (25S) were also present as nucleoprotein complexes in Triton-extracted material. Labeled DNA extracted with Triton in the form of nucleoprotein complexes was obtained in amounts which were quantitatively equivalent to the amounts extracted with deoxycholate in parallel experiments. This indicated that the newly synthesized pools of SV40 DNA may not occur as free DNA in the infected cell.

Simian virus 40 (SV40) contains closed circular deoxyribonucleic acid (DNA) molecules with superhelical turns (4, 11). A molecular explanation for the occurrence of supercoiled DNA molecules is not available at present. However, the deficiency of turns that results in the formation of a supercoiled molecule (2) may arise at the time of ring closure during the replication process because of interaction of the DNA with an organizing agent, e.g., protein.

In African green monkey kidney cells (BSC-1) infected with SV40, a nicked replicative intermediate has been described which acts as a precursor of closed circular DNA (9). The superhelix density (2) of the progeny closed circular DNA molecules may be determined by the association of such replicative intermediate molecules with protein at the time of closure. If such an association were stable, it would be possible that intracellular pools of unencapsulated SV40 DNA (5) may never be present in the free state in the infected cell.

This report presents evidence that, in BSC-1 cells infected with SV40, newly synthesized SV40 DNA can be extracted in the form of a nucleoprotein complex. After short labeling periods, the complex contains predominantly radioactive DNA with properties consistent with those of a replicative intermediate. Pulse-chase experiments indicate that such a complex

is itself a precursor of a nucleoprotein complex which contains only supercoiled SV40 DNA.

In a similar study, Green et al. (6) demonstrated a viral DNA-protein complex in mouse embryo cells infected with polyoma virus.

## MATERIALS AND METHODS

**Cell growth and virus infection.** BSC-1 cells (Flow Laboratories Ltd., Scotland) were grown in plastic petri dishes (90 by 14 mm; Nunc Ltd., Denmark) in Eagle's minimal essential medium containing 10% fetal calf serum (Biocult Ltd., Scotland) in an atmosphere of 5%  $\text{CO}_2$  in air. The wild-type strain of SV40 was plaque-purified and supplied by J. Williams, Department of Virology, Glasgow University. Confluent monolayer cultures were infected with SV40 at a multiplicity of 1 to 100 plaque-forming units per cell. After 1 hr of adsorption at 37 C, the cultures were overlaid with 10 ml of Eagle's medium containing 2% fetal calf serum.

**Enzymes.** Ribonuclease (Sigma Chemical Co., London), 200  $\mu\text{g}/\text{ml}$  in phosphate-buffered saline (PBS), was heated to 85 C for 20 min to inactivate latent deoxyribonuclease activity. Pronase (Calbiochem, Los Angeles, Calif.; 200  $\mu\text{g}/\text{ml}$  in PBS) was autodigested for 2 hr at 37 C to destroy latent deoxyribonuclease activity.

**Isolation of radioactive SV40 nucleoprotein complex.** To individual dishes of BSC-1 cells infected with SV40, 10 to 100  $\mu\text{Ci}$  of  $^3\text{H}$ -thymidine (Radiochemical Centre, Amersham, England) was added during the interval 25 to 30 hr after infection. After labeling, the

cells were washed twice with PBS, and SV40 nucleoprotein complexes were extracted by the method of Green et al. (6). To each dish, 1 ml of 0.25% Triton X-100 (British Drug Houses, Ltd.) in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 0.01 M ethylenediaminetetraacetic acid (EDTA), pH 7.9, was added and incubated at 20 C for 15 min. Sodium chloride was added to a final concentration of 0.2 M. The resulting lysate was carefully scraped into a centrifuge tube and centrifuged at 2,500 rev/min (1,500 × *g*) in a centrifuge (Measuring & Scientific Equipment, Ltd., high-speed 18) for 30 min at 4 C. The supernatant material, hereafter referred to as Triton extract, was stored at 4 C. Centrifugation at 30,000 × *g* did not significantly decrease the yield of acid-precipitable radioactivity.

The protein component of the SV40 nucleoprotein complex was labeled with <sup>14</sup>C-amino acids as follows. Dishes of monolayer BSC-1 cells were infected with SV40. After 27 hr, 50 μCi of <sup>3</sup>H-thymidine per ml and 7.5 μCi of <sup>14</sup>C-protein hydrolysate per ml (54 mCi/mAtom carbon; batch 56, from *Chlorella*; Radiochemical Centre, Amersham) were added. After 3 hr, the cells were extracted with Triton X-100 as previously described. <sup>14</sup>C-protein hydrolysate was also added in parallel to mock-infected dishes of BSC-1 cells, and Triton extracts were prepared as before.

**Preparation of labeled DNA.** A 100-μCi amount of <sup>32</sup>P-inorganic phosphate (Radiochemical Centre, Amersham) was added to single petri dishes of monolayer BSC-1 cells infected with SV40 at 20 hr after infection and thereafter at 12-hr intervals. After 3 days, SV40 DNA was extracted with sodium dodecyl sulfate (SDS) by the method of Hirt (7). The SV40 DNA extract was made 1.0 M in cesium chloride and stored at 0 C for 30 min. Cesium-SDS was removed by centrifugation at 14,000 × *g* for 10 min at 2 C. The DNA was centrifuged in 5.0 ml of cesium chloride (density, 1.52 g/ml)-propidium diiodide (500 μg/ml; Calbiochem, Los Angeles, Calif.) for 40 hr in an SW50L rotor at 40,000 rev/min at 20 C (8). The tubes were then punctured, and six-drop fractions were collected. Five-microliter portions were applied to 2.5-cm Whatman 3 MM discs and assayed for radioactivity as described below. The fractions containing supercoiled SV40 DNA were pooled and dialyzed twice against PBS for 1 hr. Propidium diiodide was removed by repeated extraction with isoamyl alcohol. Purified DNA was stored at -20 C.

Crude preparations of SV40 DNA were extracted with sodium deoxycholate from infected BSC-1 cells after labeling with <sup>3</sup>H-thymidine (3). This method extracts the intracellular pools of unencapsulated SV40 DNA.

**Centrifugation techniques and isotope measurements.** Sucrose gradient centrifugation of Triton extracts from infected cells labeled with <sup>3</sup>H-thymidine alone was performed by layering 0.2-ml samples on 4.8-ml linear sucrose gradients (5 to 20%) in 0.01 M Tris-hydrochloride, 0.2 M NaCl, 0.001 M EDTA, pH 7.9. These samples were centrifuged for 2 hr in an SW50L rotor at 36,000 rev/min in a Beckman L2-65B ultracentrifuge at 4 C and harvested as described below. Sucrose gradient centrifugation of Triton extracts from

infected cells labeled both with <sup>3</sup>H-thymidine and <sup>14</sup>C-protein hydrolysate was performed by layering 0.5-ml samples on 13.0-ml linear sucrose gradients (5 to 20%) in 0.01 M Tris-hydrochloride, 0.2 M NaCl, 0.001 M EDTA, pH 7.9. These samples were centrifuged for 3.25 hr in an SW40 rotor at 40,000 rev/min in a Beckman L2-65B ultracentrifuge at 4 C. Velocity centrifugation on cesium chloride was performed by layering 0.2-ml samples on 3.0 ml of cesium chloride (density, 1.50 g/ml). These samples were centrifuged in an SW50L rotor for 3 hr at 40,000 rev/min at 20 C and harvested as described below. Equilibrium centrifugation was performed by adding 0.2-ml samples to make a final volume of 3.2 ml of cesium chloride (density, 1.52 g/ml)-propidium diiodide (500 μg/ml). Centrifugation was carried out in an SW50L rotor for 40 hr at 40,000 rev/min at 20 C. Gradients were harvested by direct tube puncture, and two-drop fractions were collected on 2.5-cm Whatman 3 MM filter discs. The discs were washed three times in ice-cold 5% trichloroacetic acid and dried in ethanol-ether, after which they were counted in a Phillips liquid scintillation analyzer.

## RESULTS

**Isolation of SV40 nucleoprotein complex.** At 25 hr after infection of BSC-1 cells with SV40, <sup>3</sup>H-thymidine was added for 5 hr. The cells were then treated with 0.25% Triton, and a soluble supernatant extract was prepared as described above. A 0.2-ml sample of the extract was mixed with <sup>32</sup>P-labeled supercoiled (21S) SV40 DNA and centrifuged through a linear 5 to 20% sucrose gradient (Fig. 1A).

The Triton extract contained a component which sedimented more rapidly than purified <sup>32</sup>P-labeled 21S SV40 DNA and which accounted for all the radioactivity present in the sample. The rapidly sedimenting material had a sedimentation coefficient at the peak maximum of 44S, compared with 21S for the SV40 DNA marker. No <sup>3</sup>H-labeled material was detected in the region of 21S SV40 DNA. In mock-infected BSC-1 cells labeled in a similar manner, negligible amounts of acid-precipitable radioactivity were present in Triton extracts.

In an attempt to test for nonspecific association of SV40 DNA with components in the Triton extract, <sup>32</sup>P-labeled 21S SV40 DNA was incubated with a sample of Triton extract for 1 hr at 20 C. The sedimentation properties of the 21S SV40 DNA in sucrose gradients were unaltered after such treatment.

When a sample of Triton extract, mixed with <sup>32</sup>P-labeled 21S SV40 DNA, was analyzed by velocity centrifugation on cesium chloride (Fig. 1B), the <sup>3</sup>H radioactivity was found to cosediment with the <sup>32</sup>P-labeled marker DNA. This result demonstrates that the 44S material, observed in 5-hr labeled Triton extracts by sedimentation

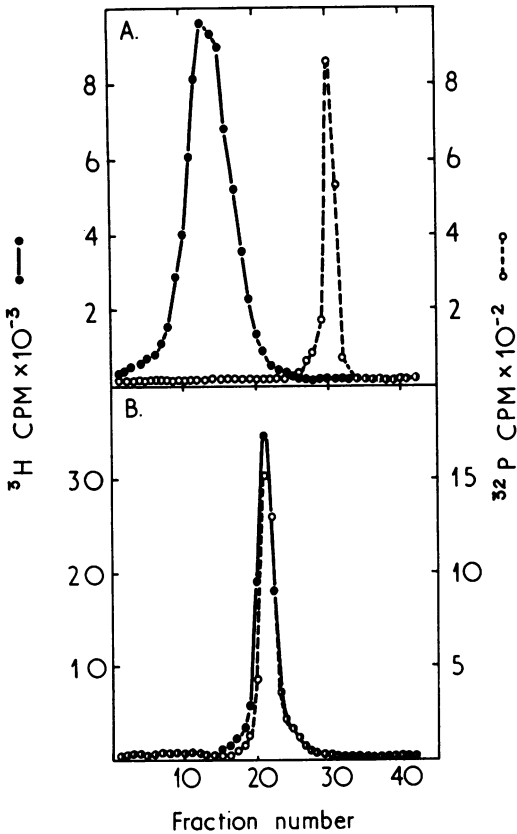


FIG. 1. Sedimentation analysis of Triton extracts from SV40-infected BSC-1 cells. After 25 hr, the cells were labeled with  $^3\text{H}$ -thymidine for 5 hr before harvesting.  $^{32}\text{P}$ -labeled 21S SV40 DNA was added as marker. (A) Centrifugation on a 5 to 20% sucrose gradient was for 2 hr in an SW50L rotor at 36,000 rev/min at 4 C. (B) Sample was layered on 3 ml of cesium chloride (density, 1.50 g/ml) and centrifuged for 3 hr in an SW50L at 40,000 rev/min at 20 C.

analysis on sucrose gradients, contains 21S SV40 DNA, which is associated with other macromolecular components.

**Evidence of protein in the complex.** To determine the nature of the material complexed to SV40 DNA in the 44S component, the experiments described below were performed. A 0.2-ml sample of the Triton extract, obtained in the previous experiment, was incubated with 10  $\mu\text{g}$  of heat-treated ribonuclease per ml for 15 min at 20 C. Another sample of the Triton extract was incubated with 10  $\mu\text{g}$  of Pronase per ml under the same conditions. Each sample was mixed with  $^{32}\text{P}$ -labeled 21S SV40 DNA and centrifuged through 5 to 20% sucrose gradients as previously described (Fig. 2).

Digestion of the complex with Pronase under the conditions described gave rise to a component

(Fig. 2A) with a sedimentation coefficient of 39S. More prolonged incubation led to a further reduction in sedimentation coefficient.

Incubation of the complex with ribonuclease (Fig. 2B) did not decrease the rate of sedimentation of the complex. It is therefore evident that the complex did not contain significant amounts of ribonucleic acid. A slight increase in the average sedimentation coefficient (48S) was observed and is attributed to association of the complex with ribonuclease. A similar effect has been reported by Green et al. (6).

Double-label experiments were performed with  $^3\text{H}$ -thymidine and  $^{14}\text{C}$ -protein hydrolysate to provide additional evidence that the material associated with the viral DNA was protein (Fig. 3). BSC-1 cells infected with SV40 were labeled 27 hr after infection with  $^3\text{H}$ -thymidine and  $^{14}\text{C}$ -protein hydrolysate for 3 hr, and Triton extracts were prepared. Samples of 0.5 ml were centrifuged

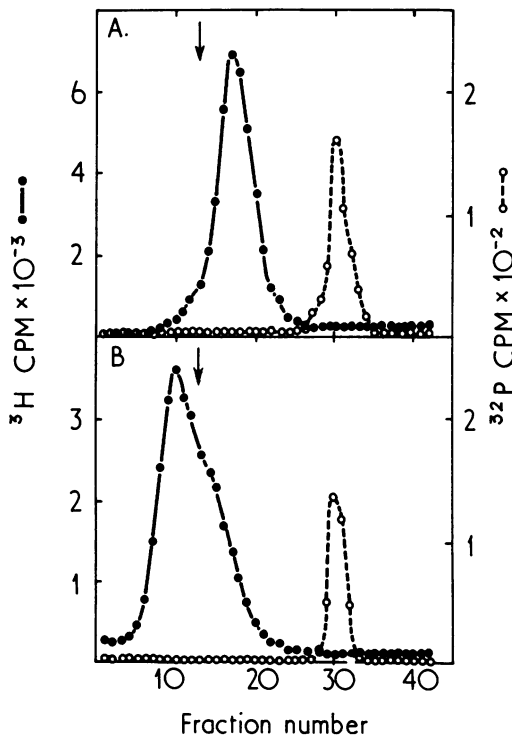


FIG. 2. (A) Sedimentation of Triton extract after incubation with Pronase, which was added to a final concentration of 10  $\mu\text{g}/\text{ml}$ ; the mixture was incubated for 15 min at 20 C. (B) Sedimentation of Triton extract after treatment with ribonuclease, which was added to a final concentration of 10  $\mu\text{g}/\text{ml}$ ; the mixture was incubated for 15 min at 20 C. Procedures for preparation of Triton extract and for sucrose gradient analysis are described in Fig. 1. Arrow indicates the position of the complex in untreated Triton extract.

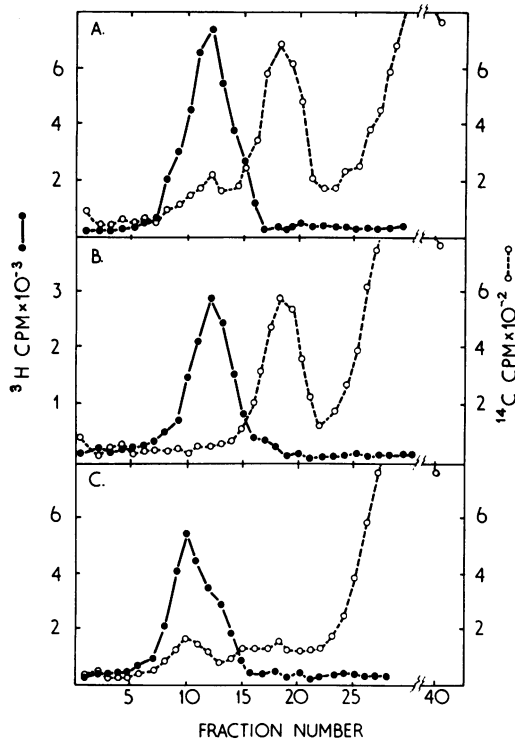


FIG. 3. Sedimentation of Triton extracts from SV40-infected BSC-1 cells and from mock-infected BSC-1 cells after labeling with  $^{14}\text{C}$ -protein hydrolysate. (A) Cells 27 hr after infection were labeled for 3 hr with  $^{14}\text{C}$ -protein hydrolysate and  $^3\text{H}$ -thymidine, and Triton extracts were prepared. (B) Mock-infected BSC-1 cells were labeled in parallel with  $^{14}\text{C}$ -protein hydrolysate, and Triton extracts were prepared. (C) The double-labeled extract from infected cells described above was incubated with  $20\ \mu\text{g}$  of ribonuclease per ml for 1 hr at  $37\ \text{C}$ . All samples were centrifuged in 5 to 20% linear sucrose gradients for 3.25 hr in an SW40 rotor at 40,000 rev/min in a Beckman L2-65B ultracentrifuge at  $4\ \text{C}$ . Only the first 30 fractions out of the 40 collected are shown, because of the large amount of  $^{14}\text{C}$ -labeled material ( $\sim 10,000$  counts/min) at the top of the gradient.

through a linear 5 to 20% sucrose gradient, the results are shown in Fig. 3A. A small amount of  $^{14}\text{C}$ -labeled material was observed to cosediment with  $^3\text{H}$ -labeled complex. Also, a  $^{14}\text{C}$ -labeled component with a sedimentation coefficient of 40S was observed. A large amount of  $^{14}\text{C}$ -labeled material, probably free protein, remained at the top of the gradient. Triton extracts of mock-infected BSC-1 cells similarly labeled with  $^{14}\text{C}$ -protein hydrolysate were prepared, and a 0.5-ml portion was mixed with 0.1 ml of Triton extract from infected cells labeled with  $^3\text{H}$ -thymidine as shown in Fig. 1. The mixture was centrifuged on a linear 5 to 20% sucrose gradient, and the results

are shown in Fig. 3B. A  $^{14}\text{C}$ -labeled 40S component, along with a large amount of  $^{14}\text{C}$ -labeled material, was observed at the top of the gradient. We conclude that this 40S component (Fig. 3A) probably is of cellular origin and does not result from virus infection. As preliminary labeling experiments with  $^3\text{H}$ -uridine indicated that the 40S component contained ribonucleic acid, 0.5-ml samples of the Triton extract analyzed in Fig. 3A were incubated with  $20\ \mu\text{g}$  of ribonuclease per ml for 1 hr at  $37\ \text{C}$  and analyzed on linear 5 to 20% sucrose gradients (Fig. 3C). The 40S component observed in Fig. 3A was no longer present, but the  $^{14}\text{C}$ -labeled material associated with the  $^3\text{H}$ -labeled 44S complex was unaffected by this treatment. The 40S component probably is a ribonucleoprotein of cellular origin whose presence in Triton extracts is independent of SV40 infection. The 44S  $^{14}\text{C}$ -labeled material occurs only in SV40-infected cells and probably is due to protein associated with the viral DNA. The nature of the protein is under investigation. These results indicate that, in the complex extracted with Triton, 21S SV40 DNA probably is associated with protein.

The effect of salt concentration and detergent treatment on the sedimentation properties of the nucleoprotein complex is shown in Fig. 4. When the Triton extract was treated with 0.5 M NaCl, a component was observed on sucrose gradients with a sedimentation coefficient of 29S (Fig. 4A). Treatment with either 1.0 M NaCl (Fig. 4B) or 0.5% SDS (Fig. 4C) yielded components which sedimented only slightly in advance of the 21S SV40 DNA marker, indicating effectively complete dissociation of the complex. Binding of protein to DNA in the complex is sensitive to variation in salt concentration; this behavior is similar to that of the polyoma DNA-protein complex in infected mouse embryo cells (6). Experiments are in progress to obtain quantitative information for such DNA-protein interactions in the presence of salt.

**Detection of replicating SV40 DNA as a nucleoprotein complex.** Levine et al. (9) demonstrated that when SV40-infected African green monkey kidney cells were exposed to  $^3\text{H}$ -thymidine for periods of about 5 min, little or no radioactivity was present as 21S SV40 DNA. Instead, a component with the properties of a replicative intermediate DNA molecule was present and had a sedimentation coefficient of 25S.

In view of these results, short-term labeling experiments were performed with SV40-infected BSC-1 cells to determine the nature of such replicating DNA molecules in Triton extracts. Six monolayer cultures of BSC-1 cells were infected with SV40 virus, and after 30 hr the medium was

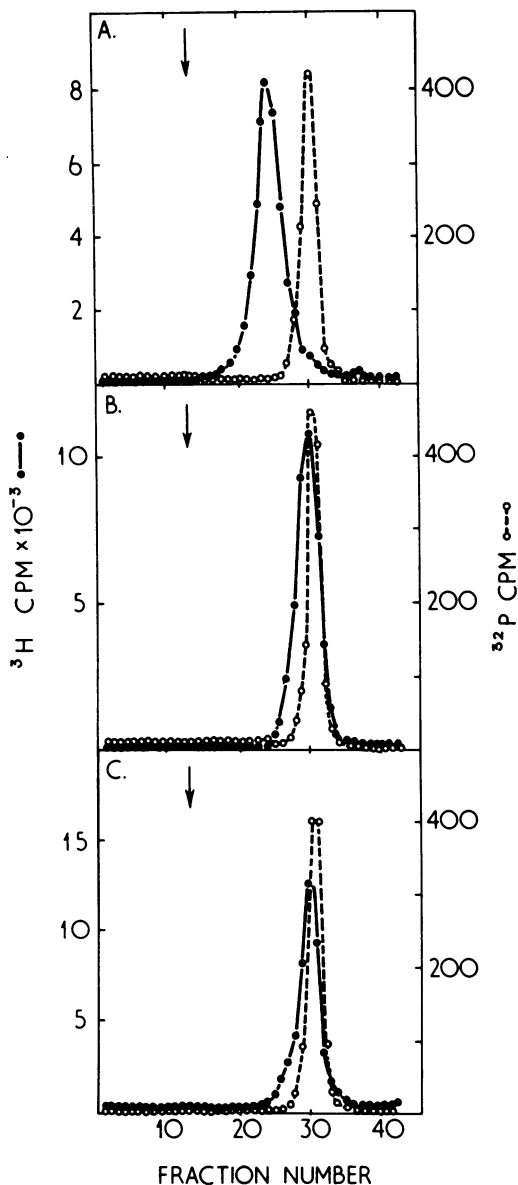


FIG. 4. Sedimentation of Triton extracts after treatment in dissociating conditions. (A) Triton extract was adjusted to 0.5 M in sodium chloride and incubated for 15 min at 20 C. Sample was centrifuged in a 5 to 20% sucrose gradient adjusted to 0.5 M in sodium chloride. (B) Triton extract was adjusted to 1.0 M in sodium chloride and incubated for 15 min at 20 C. The sample was centrifuged in a 5 to 20% sucrose gradient adjusted to 1.0 M in sodium chloride. (C) Triton extract was made 0.5% with respect to sodium dodecyl sulfate and incubated for 1 hr at 37 C. The sample was centrifuged in a 5 to 20% sucrose gradient. The Triton extract was prepared from SV40-infected BSC-1 cells as described in Fig. 1.  $^{32}\text{P}$ -labeled 21S SV40 DNA was added as marker, and centrifugation was for 2 hr in an SW50L rotor at 36,000 rev/min at 4 C. Arrow indicates the position of the complex in untreated Triton extract.

removed and replaced with 2 ml of Eagle's medium containing 80  $\mu\text{Ci}$  of  $^3\text{H}$ -thymidine per ml in the absence of serum. After 5 min of incubation at 37 C, the radioactive medium was removed. The cells were washed with PBS, and four of the dishes were overlaid with 10 ml of Eagle's medium containing 500  $\mu\text{g}$  of unlabeled thymidine per ml and incubated at 37 C. The fifth dish was extracted with 0.25% Triton, and the sixth was extracted with 0.25% deoxycholate, which extracts intracellular pools of unencapsulated SV40 DNA (3, 5). After a 5-min chase, the first two dishes were extracted, one with Triton and the other with deoxycholate as described previously. After a 15-min chase, the remaining dishes were extracted similarly.

Samples (0.2 ml) of the three Triton extracts were analyzed by sucrose gradient centrifugation to detect the presence of nucleoprotein complexes and by velocity analysis on cesium chloride to characterize the DNA species present. The deoxycholate extracts also were analyzed by cesium chloride velocity centrifugation. Analysis of the Triton extracts on sucrose gradients is shown in Fig. 5.

In cells labeled with  $^3\text{H}$ -thymidine for 5 min, the Triton extract contained material with a broad sedimentation distribution profile (45S to 60S) (Fig. 5A). Samples in the regions indicated by the horizontal bars were pooled to determine the type of DNA present in these parts of the gradient. In Triton extracts obtained after a 5-min chase period (Fig. 5B), the proportion of material with high S values was diminished, but a significant amount sedimented faster than the 44S component in the form of a diffuse leading boundary. After a 15-min chase period (Fig. 5C), the Triton extract contained a population of molecules which appeared much less polydisperse and consisted mainly of 44S material. These results indicate progressive conversion of rapidly sedimenting material to the 44S component. Material with sedimentation properties of 25 or 21S SV40 DNA was not observed in any significant amount when the Triton extracts were analyzed on sucrose gradients. It is therefore possible that the intracellular pools of SV40 DNA may occur not in the free state, but rather in association with protein.

The properties of DNA present in the Triton extracts were analyzed by cesium chloride velocity centrifugation and by means of cesium chloride-propidium diiodide gradients at equilibrium (Fig. 6).

These results demonstrate that, after 5 min of exposure to  $^3\text{H}$ -thymidine, a large proportion of the DNA in the Triton extract was in the form of 25S SV40 DNA (Fig. 6A). The 25S DNA was progressively chased into 21S SV40 DNA (Fig. 6B and 6C). An essentially similar sequence of

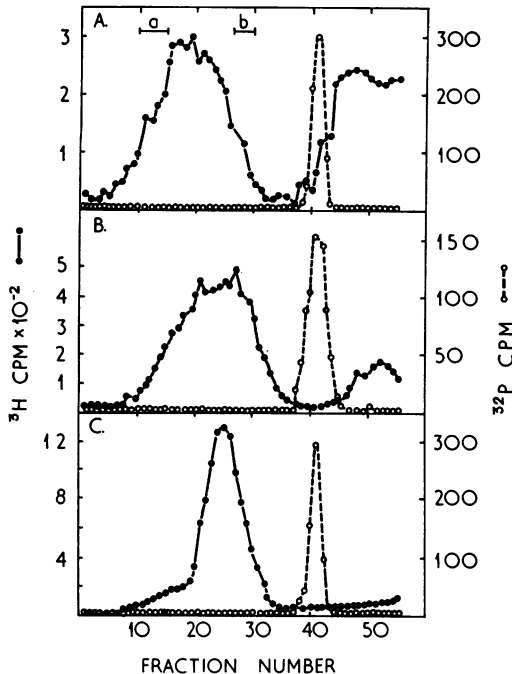


FIG. 5. Sedimentation of Triton extracts from SV40-infected BSC-1 cells exposed to  $^3\text{H}$ -thymidine and then chased with cold thymidine. Cells 30 hr after infection were labeled for 5 min (A) and then chased with cold thymidine for 5 min (B) and for 15 min (C).  $^{32}\text{P}$ -labeled 21S SV40 DNA was added as marker, and samples were centrifuged in 5 to 20% sucrose gradients for 2 hr in an SW65 rotor at 36,000 rev/min at 4 C. Horizontal bars a and b in A represent fractions pooled for subsequent analysis of DNA species present in these regions.

events was observed in the SV40 DNA extracted with deoxycholate, in agreement with the observations of Levine et al. (9). However, the low-molecular-weight DNA observed in short labeling and chase periods in these experiments with BSC-1 cells (Fig. 6A and 5A) were not reported in SV40-infected African green monkey kidney cells (9). We cannot at present exclude the possibility that this small DNA chases into 21S viral DNA. Alternatively, the small DNA may arise from the host cell; hybridization experiments are necessary to investigate this point.

Analysis of the samples by means of cesium chloride-propidium diiodide gradients at equilibrium demonstrated clearly the progressive conversion of 25S DNA to 21S DNA with increasing time of chase with cold thymidine (Fig. 6D, 6E, and 6F). It was observed (Fig. 6D) that the peak of replicating SV40 DNA was present as a broad heterogeneous distribution which extended to the 21S DNA marker position in a manner similar to

that reported by Sebring and co-workers (Sebring et al., Fed Proc. 30: 1177, 1971). The distribution became more clearly resolved after 5- and 15-min chases with cold thymidine. This improved resolution allowed accurate quantitation of the percentage of 21S SV40 DNA present at each time interval, and a comparison is drawn with DNA extracted from infected cells with deoxycholate at corresponding times (Table 1). These results indicate that essentially the same proportion of 21S SV40 DNA was present at corresponding times in the Triton and deoxycholate extracts. Further experiments to estimate the efficiency of extraction of acid-precipitable radioactivity demonstrated that the two extraction procedures gave the same yield of DNA (within 5%). We exclude, on this basis, the possibility of selective extraction of nucleoprotein complexes with Triton; i.e., the DNA extracted in the form of complexes by means of Triton represents at least the major portion of the DNA extractable with deoxycholate. Replicating form SV40 DNA appeared to be present only when complex was present with sedimentation coefficient greater than 44S. To confirm this finding and to test for the presence of 21S viral DNA throughout the heterogeneous distribution indicated in Fig. 5A, the regions indicated by the horizontal bars were analyzed on cesium chloride-propidium diiodide equilibrium gradients (Fig. 7). Rapidly sedimenting material from the region indicated in Fig. 5A (bar a) contained only replicating-form DNA (Fig. 7A), but only covalently closed SV40 DNA was present at the trailing edge (Fig. 5A, bar b) of the population (Fig. 7B).

## DISCUSSION

The experiments reported here show that, when SV40-infected BSC-1 cells are exposed to radioactive thymidine, all forms of labeled SV40 DNA can be extracted in the form of virus DNA-protein complexes. After labeling for long periods with  $^3\text{H}$ -thymidine, a 44S complex was present which contained only 21S SV40 DNA. When shorter pulse times were used, we found, in addition to 44S material, protein-DNA complexes with higher S values which contained replicative form SV40 DNA. In a similar study, Green et al. (6) demonstrated that polyoma DNA-protein complexes can be extracted from infected mouse embryo cells. In our experiments, free DNA was never observed with the Triton extraction procedure.

Our conclusion that the altered sedimentation characteristics of the forms of SV40 DNA in the infected cell result from protein binding is based principally on the sensitivity of the complex to

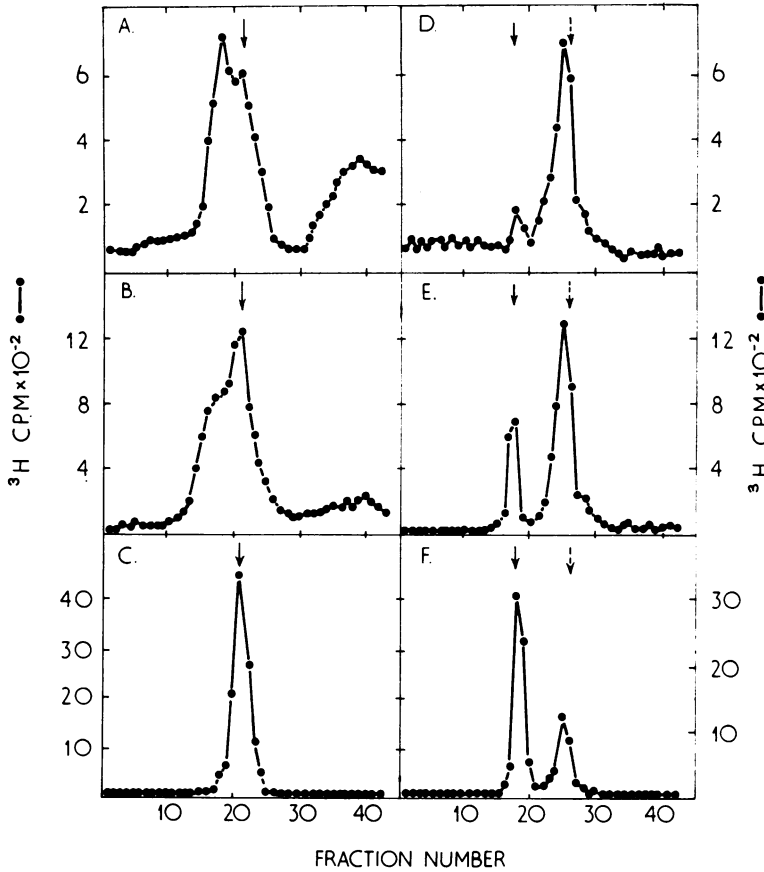


FIG. 6. Analysis of Triton extracts by cesium chloride velocity centrifugation and cesium chloride-propidium diiodide buoyant centrifugation after exposure to  $^3\text{H}$ -thymidine and subsequent chase with cold thymidine. Three dishes of cells 30 hr after infection were labeled with  $^3\text{H}$ -thymidine for 5 min. One dish was extracted with Triton for velocity analysis (A) and buoyant analysis (D). The remaining dishes were washed free from isotope, and medium containing cold thymidine was added. After a 5-min chase, one dish was extracted with Triton for velocity analysis (B) and buoyant analysis (E). After a 15-min chase, the remaining dish was extracted with Triton for velocity analysis (C) and buoyant analysis (F). For velocity centrifugation, samples were mixed with  $^{32}\text{P}$ -labeled 21S SV40 DNA and layered on 3 ml of cesium chloride (density, 1.50 g/ml). Centrifugation was performed for 3 hr in an SW50L rotor at 40,000 rev/min at 20 C. Arrow indicates position of 21S marker DNA. For buoyant analysis in cesium chloride-propidium diiodide gradients, samples were mixed with  $^{32}\text{P}$ -labeled 21 and 16S SV40 DNA and centrifuged in cesium chloride (density, 1.52 g/ml)-propidium diiodide (500  $\mu\text{g}/\text{ml}$ ) for 40 hr in an SW-50L rotor at 40,000 rev/min at 20 C. Arrows indicate the positions of the marker DNA species.

protease digestion and on labeling studies with  $^{14}\text{C}$ -labeled amino acids. Interpretation of the protein labeling experiments was complicated by the presence of radioactive ribonucleoproteins of cellular origin. However, after digestion with ribonuclease, a virus-specific component labeled with  $^{14}\text{C}$ -amino acids was more clearly evident in a position corresponding to that of  $^3\text{H}$ -labeled SV40 DNA in double-label experiments. The double-label experiments do not conclusively demonstrate, however, that the labeled protein in the 44S component is bound to the viral DNA; it is

TABLE 1. Percentage of 21S SV40 DNA in Triton and deoxycholate extracts

Time of chase <sup>a</sup> (min)	Total counts/min in 21S SV40 DNA (%)	
	Triton extract	Deoxycholate extract
0	14	17
5	26	24
15	71	66
55	100	100

<sup>a</sup> After 5 min of exposure to  $^3\text{H}$ -thymidine.

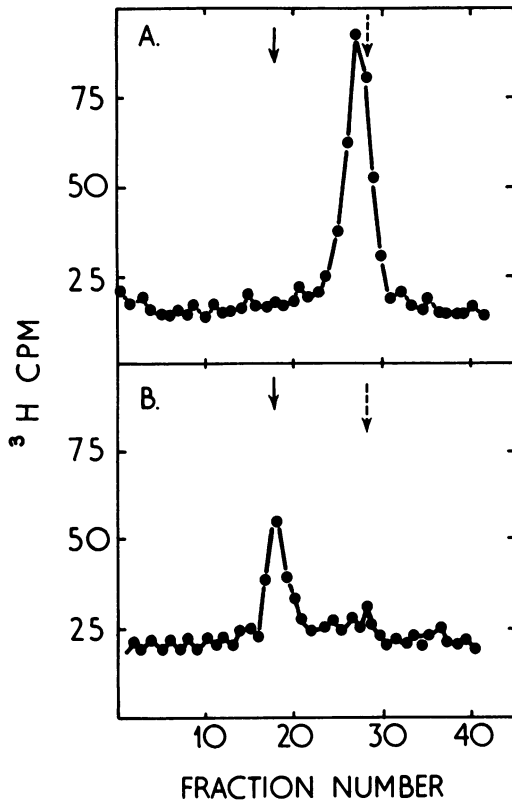


FIG. 7. Buoyant behavior of material fractionated from sucrose gradients of Triton extracts from SV40-infected BSC-1 cells after exposure to  $^3\text{H}$ -thymidine for 5 min. Fractions were prepared from the experiment described in Fig. 5A. Pooled samples indicated by horizontal bars *a* and *b* were dialyzed free from sucrose, adjusted to a density of 1.52 g/ml with cesium chloride and to 500  $\mu\text{g}/\text{ml}$  with propidium diiodide, and centrifuged to equilibrium as described in the legend to Fig. 6. (A) Material from pooled samples indicated by bar *a* in Fig. 5A. (B) Material from the pooled samples indicated by bar *b* in Fig. 5A.

possible that the proteins represent some other virus-induced component which fortuitously co-sediments with the  $^3\text{H}$ -labeled complex. This possibility could be explored by means of alternative purification and fractionation techniques. Indeed, the properties of the complexes which we report may depend largely on the extraction conditions employed. To demonstrate that, because of nonspecific binding of protein to virus DNA, the complexes do not appear in the course of extraction, reconstruction experiments were performed in which purified 21S SV40 DNA was incubated with Triton extracts. The sedimentation properties of the virus DNA were unaltered by such treatment, and no complexes were present. Because these experiments cannot duplicate

exactly the conditions in the cell at the time of extraction, we cannot exclude the possibility of some form of nonspecific interaction.

Nucleoprotein complex with a sedimentation coefficient of 44S contained 21S SV40 DNA in amounts quantitatively equivalent to that extracted with deoxycholate in parallel experiments. On treatment with sodium chloride, this complex showed properties similar to those of polyoma DNA-protein complexes (6). It is not known whether this complex plays a part in SV40 virion assembly or whether the protein is specified by the host cell or by the virus itself. However, the complex may be related to SV40 core complex (1) which has a sedimentation coefficient of 46S and is dissociated by high salt concentration.

Short-term labeling experiments indicated the presence of nucleoprotein complexes which contained replicative-form DNA. The properties and kinetics of conversion of this 25S DNA to 21S DNA are compatible with the results reported in African green monkey kidney cells infected with SV40 (9) and in polyoma-infected mouse embryo cells (3). The 25S SV40 DNA present in rapidly sedimenting complexes could be chased into the 21S SV40 DNA present in the 44S material.

Precise analysis of the sedimentation distributions obtained for the nucleoprotein complexes is difficult. Changes in sedimentation behavior could arise from changes in molecular weight or conformation, or both.

The association with protein could have important consequences in terms of the superhelix density of the closed circular daughter molecules. If different amounts of protein or a variable number of types of protein were to associate with 25S SV40 DNA, it might be possible to account for the superhelix density heterogeneity which exists in intracellular 21S SV40 DNA in infected cells (5).

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