Nucleoprotein Complexes Containing Replicating Simian Virus 40 DNA: Comparison with Polyoma Nucleoprotein Complexes

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Procedures for isolating nucleoprotein complexes containing replicating polyoma DNA from infected mouse cells were used to prepare short-lived nucleoprotein complexes (r-SV40 complexes) containing replicating simian virus 40 (SV40) DNA from infected monkey cells. Like the polyoma complexes, r-SV40 complexes were only partially released from nuclei by cell lysis but could be extracted from nuclei by prolonged treatment with solutions containing Triton X-100. r-SV40 complexes sedimented faster than complexes containing SV40 supercoiled DNA (SV40 complex) in sucrose gradients, and both types of SV40 nucleoprotein complexes sedimented ahead of polyoma complexes containing supercoiled polyoma DNA (py complex). The sedimentation rates of py complex and SV40 complex were 56 and 61S, respectively, based on the sedimentation rate of the mouse large ribosomal subunit as a marker. r-SV40 complexes sedimented as multiple peaks between 56 and 75S. Sedimentation and buoyant density measurements indicated that protein is bound to all forms of SV40 DNA at about the same ratio of protein to DNA (1-2/1) as was reported for polyoma nucleoproteins.

Nucleoprotein complexes containing newly replicated viral DNA can be extracted from polyoma- and simian virus 40 (SV40)-infected cells after lysis with nonionic detergents (8, 10, 23). Complexes isolated after long pulses of ³H-thymidine contain mostly supercoiled viral DNA, while complexes isolated after short pulses (5-30 min) contain both supercoiled and replicating viral DNA. Many properties of polyoma supercoiled DNA-protein complexes (py complex) are similar to the corresponding properties of SV40 supercoiled DNA-protein complexes (SV40 complex), yet the reported sedimentation coefficients differ by more than 20% (8, 10, 23).

Replicating viral DNA-protein complexes appear to be precursors to supercoiled viral DNA-protein complexes (8, 23). In the preceding communication some properties of the polyoma complexes containing replicating DNA (r-py complex) were described (8). In this study, we have used the same methods to isolate complexes containing replicating SV40 DNA (r-SV40 complexes) from infected CV-1 cells and have compared the properties of these complexes to those of r-py complexes.

MATERIALS AND METHODS

Virus and cells. Strain RH 911 SV40 virus was grown in the TC7 subline of CV-1 cells (17). Cells were propagated in 9-cm plastic petri dishes (A. S. Nünc) at 37 C. Monolayers were grown to confluence in Eagle medium supplemented with 10% calf serum. Cultures were infected 24 to 48 h after confluence, and the virus was allowed to absorb for 90 min. After absorption, 4 ml of medium containing 5% horse serum was added to each culture. The methods for cultivation, isotope labeling, and purification of polyoma virus have been described (8, 15).

Triton extraction of SV40-infected cells. SV40 nucleoprotein complexes were extracted by procedures described in the preceding paper (8). Confluent monolayers were pulsed with ³H-thymidine (55 Ci/mmol) at 40 h after infection for 2 h (10 μ Ci/ml) or at 41.75 h for 15 min (100 μ Ci/ml). After pulses, monolayers were washed twice with ice-cold Tris-buffered saline containing magnesium and calcium (TBS) (24). Each petri plate was then treated with 0.9 ml of lysing fluid (TTE) containing 0.25% Triton X-100, 0.01 M Tris-hydrochloride (pH 7.9), and 0.01 M EDTA (10). Cells were allowed to swell for 10 min at 22 C, and 0.1 ml of 2.0 M NaCl was added to each plate. Although addition of NaCl caused the cells to lyse, nuclei remained morphologically intact and formed large aggregates. The lysate, containing nu-

clear aggregates, was decanted or gently scraped from the plates with a rubber policeman and poured into ice-cold, 12-ml centrifuge tubes. Nuclear aggregates and large cellular debris were pelleted by centrifugation at 2,000 rpm for 5 min at 4°C in the HL-4 swinging bucket rotor of a Sorvall GLC centrifuge. The supernatant fluid (Triton supernatant fraction) was decanted and stored at 4°C. The SV40 DNA-protein complex in this fraction was designated Triton supernatant complex (TSC).

The pellet obtained from low-speed centrifugation of the Triton cell lysate was resuspended in ice-cold TTE (0.9 ml/petri dish), one-tenth volume of 2.0 M NaCl was added, and the solution was incubated for 3 h on ice with occasional mixing. Nuclei remained intact and were pelleted at 2,000 rpm as described above. The supernatant fluid (Triton nuclear fraction) was decanted and stored at 4°C. The SV40 nucleoprotein complex in the nuclear fraction was designated nuclear extraction complex (NEC).

¹⁴C-labeled py complex. Labeled polyoma nucleoprotein complex (py complex) was extracted from cells pulsed for 6 h with ¹⁴C-thymidine (1 µCi/ml) 22 h after infection and lysed with Triton as described above. Py complex in the Triton supernatant fraction was used as a marker for sedimentation coefficients and density determinations throughout this investigation. We have previously shown that glutaraldehyde-fixed py complex has a density in CsCl of 1.437 g/cm³, and 85 to 90% of the DNA in the complex is supercoiled (8).

Velocity sedimentation. Linear 5 to 20% (wt/wt) sucrose gradients were prepared in TTE buffer containing 0.2 M NaCl (STTE). Samples (0.1 or 0.2 ml) were layered onto 4.8-ml gradients and centrifuged at 36,000 rpm for 120 min at 4°C in a Spinco SW50.1 rotor (10). Fractions were collected, and trichloroacetic acid-precipitable radioactivity was determined (15). For velocity sedimentation in CsCl, a 0.2-ml sample of DNA was layered on 3.0 ml of a CsCl solution (density 1.50 g/cm³ in 0.01 M EDTA, pH 7.5) and centrifuged in a Spinco SW50.1 rotor at 25°C for 3.0 h at 35,000 rpm.

Buoyant density centrifugation of glutaraldehyde-fixed complexes. Peak fractions of SV40 and polyoma nucleoprotein complexes pooled from sucrose gradients were fixed with glutaraldehyde as previously described (8). The density of glutaraldehyde-fixed samples of polyoma and SV40 nucleoprotein complexes was determined by analysis in preformed linear CsCl gradients (density 1.3 g/cm³ to 1.6 g/cm³), prepared and centrifuged as previously described (8).

Dye-buoyant density gradient centrifugation. Analysis of DNA by dye-buoyant density centrifugation in CsCl-propidium diiodide (CsCl-PI) density gradients was described in the preceding report (8).

Benzoylated naphthoylated DEAE-cellulose (BND) chromatography. Portions of ³H-labeled Triton nuclear and Triton supernatant fractions (0.5 to 1.0 ml) were diluted to a volume of 2.7 ml in 0.2 M Tris-hydrochloride (pH 8.0), 0.01 M EDTA. A 0.3-ml amount of 10% SDS was added, and the solution was incubated at 50°C for 20 min. After cooling to room temperature, NaCl was added to a final concentration

of 0.5 M, and the DNA was extracted by mixing the solution with an equal volume of redistilled phenol saturated with 0.2 M Tris-hydrochloride (pH 8.0), 0.01 M EDTA for 15 min at room temperature. After low-speed centrifugation, the phenol phase was discarded, yeast tRNA was added to the aqueous phase as carrier, and the DNA was precipitated by adding 2 vol of absolute ethanol at -20°C overnight. DNA was suspended in 1 ml of 0.01 M Tris-hydrochloride (pH 7.2), 0.001 M EDTA (Tris-EDTA) in 0.3 M NaCl and applied to a 0.9- by 30-cm Sephadex (Pharmacia) G-50 column equilibrated with the same buffer at room temperature. The ³H-labeled DNA peak was pooled (6-7 ml) and adsorbed to a 0.9- by 10-cm BND-cellulose (Gallard-Schlesinger) column equilibrated in 0.3 M NaCl, Tris-EDTA buffer (5, 14). After washing the column with 2 vol of the same buffer, double-stranded DNA was eluted by passing 1.0 M NaCl, Tris-EDTA buffer through the column at a rate of 0.5 ml/min. An additional 40 ml of 1.0 M NaCl, Tris-EDTA buffer was passed through the column, and then replicating DNA was eluted with 1 M NaCl, Tris-EDTA buffer containing 2% caffeine. The column was washed with an additional 20 ml of the 1 M NaCl Tris-EDTA buffer containing 2% caffeine, followed by a final 20-ml wash with 0.3 M NaCl, Tris-EDTA buffer containing 4 M urea.

RESULTS

Triton extraction of nucleoproteins from SV40-infected cells. SV40-infected CV-1 cells were pulsed with ³H-thymidine for 15 or 120 min about 40 h after infection. Cells were lysed with Triton X-100 and separated into supernatant and nuclear pellet fractions. Nuclei were resuspended in buffer containing Triton and SV40 nucleoprotein complex were extracted for 3 h at 0°C. After extraction, nuclei were recentrifuged and low-molecular-weight DNA was extracted from the resulting pellet by a modification of the Hirt SDS selective extraction procedure (8, 12). The sum of the radioactivity in these three cell fractions was about equal to the radioactivity obtained by Hirt extraction of total viral DNA (8).

Samples were withdrawn from each sample, and the alkali-resistant, trichloroacetic acid-precipitable radioactivity was measured (Table 1). After a 15-min pulse, the Triton supernatant fraction contained only about half of the total radioactive viral DNA, while the remainder was associated with the nuclear fraction. About 70% of the viral DNA remaining in the nuclear fraction could be extracted by prolonged incubation of nuclei with Triton lysing buffer. After a 2-h pulse, however, the majority of radioactive viral DNA was found in the Triton supernatant fraction while only an additional 14% was reextracted from nuclei.

Portions from each extract were mixed with ¹⁴C-thymidine-labeled py complex, and the

TABLE 1. *Distribution of SV40 nucleoprotein complexes in subcellular fractions^a*

Length of pulse (min)	Fraction (% of total)		
	Triton supernatant	Triton ^b nuclei	SDS ^c pellet
15	57	30	13
120	78	14	8

^a The values are based on alkali-resistant, trichloroacetic acid-precipitable radioactivity (8).

^b Nuclei obtained by Triton lysis were extracted by incubation for 3 h in STTE buffer.

^c Nuclei extracted in STTE buffer were recentrifuged and extracted by a modified Hirt procedure (8).

samples were analyzed in 5 to 20% sucrose gradients (Fig. 1). In all gradients SV40 nucleoprotein complexes sedimented ahead of py complex. SV40 nucleoprotein complex extracted in the Triton supernatant fraction (TSC) after a 120-min pulse sedimented as a homogeneous component only slightly ahead of ¹⁴C-py complex (Fig. 1A). A similar pattern was observed for the nucleoprotein complex obtained by Triton extraction of nuclei (NEC) after the 120-min pulse (not shown in Fig. 1).

In contrast, TSC prepared from cells pulsed for 15 min sedimented as two peaks, one corresponding to the TSC peak extracted after the 120-min pulse and a smaller peak sedimenting about 15% faster (Fig. 1B). The SV40 nucleoprotein complex obtained by Triton extraction of nuclei (NEC) pulsed for 15 min (Fig. 1C) also sedimented as two peaks, but both peaks were faster than the TSC peak from the 120-min pulse and corresponded more closely with the fast peak in the 15-min TSC.

The results presented above are similar to those obtained with polyoma-infected mouse cells (8). Triton lysis of cells after a short pulse liberates into the supernatant fraction a mixture of fast sedimenting polyoma nucleoprotein complexes containing replicating viral DNA and slower sedimenting complexes containing both replicating viral DNA and supercoiled viral DNA. Complexes extracted from the nuclear fraction are mostly fast sedimenting and contain replicating viral DNA. With long pulses, relatively small amounts of fast sedimenting complexes containing replicating DNA are found and most of the complexes extracted into the Triton supernatant fraction contain supercoiled viral DNA.

Characterization of the DNA in SV40 nucleoprotein complexes. To determine whether SV40 complexes did indeed contain a similar distribution of supercoiled and replicating DNA

as that found after polyoma infection, Triton supernatant and Triton nuclear fractions were treated with SDS to strip the protein from complexes, and the DNA was analyzed by velocity sedimentation, equilibrium centrifugation in CsCl-PI density gradients and by chro-

FIG. 1. Velocity sedimentation in 5 to 20% sucrose gradients of polyoma and SV40 nucleoprotein complexes. SV40-infected cultures were either labeled with ³H-thymidine (10 μ Ci/ml) for 2 h at 40 h after infection or for 15 min (100 μ Ci/ml) at 41.75 h after infection. SV40 nucleoprotein complexes were prepared from Triton supernatant (TSC) and Triton nuclear (NEC) fractions. Polyoma nucleoprotein complex (TSC) was prepared from cells pulsed for 6 h with ¹⁴C-thymidine (1 μ Ci/ml) 22 h after infection and lysed with Triton. ¹⁴C-labeled polyoma TSC mixed with ³H-labeled SV40 TSC from a 2-h pulse (A), and ³H-labeled SV40 TSC (B), or NEC from a 15-min pulse (C).

matography on BND-cellulose. In velocity gradients, the Triton supernatant fraction prepared after a 15-min pulse contained a large peak of 21S DNA, whereas 25S DNA (replicating SV40 DNA) was distributed about equally between the Triton supernatant fraction and the Triton nuclear fraction (Fig. 2A and B, Table 1). In contrast, after a 120-min pulse, most labeled material in both the Triton supernatant fraction and the Triton nuclear fraction was 21S viral DNA (Fig. 2C and D). The nuclear extract prepared after a chase (Fig. 2D) does, nevertheless, contain a small amount of SV40 25S DNA. However, the 25S DNA in this nuclear fraction only represents about 5% of the total labeled viral DNA (see also Table 1).

Analysis of DNA in CsCl-PI equilibrium density gradients clearly shows a differential extraction of complexes containing supercoiled and replicating DNA between the Triton supernatant and Triton nuclear fractions after a 15-min pulse (Fig. 3A and B). About 40% of the DNA in the Triton supernatant formed a "heavy" peak characteristic of supercoiled viral DNA while the rest banded at intermediate and light densities characteristic of replicating and

nicked DNA (8, 13, 19). However, very little supercoiled DNA was found in the Triton nuclear fraction and almost 90% of the DNA in this fraction had buoyant densities characteristic of replicating DNA (Fig. 3B). Actually, the supercoiled DNA in the Triton nuclear fraction represented only about 9% of the total ^3H -labeled supercoiled DNA (refer also to Table 1). As expected, after a 120-min pulse, most of the DNA in the supernatant as well as the nuclear fraction is supercoiled (Fig. 3C and D).

Comparison of the DNA in velocity gradients (Fig. 2) and in CsCl-PI gradients (Fig. 3) reveals that about half of the DNA sedimenting in the 21S region of the Triton supernatant fraction after a 15-min pulse is actually SV40 replicating DNA (SV40 RI DNA). Although a large amount of radioactivity in the Triton nuclear fraction also sediments in the 21S region of the gradient, almost all of this DNA is replicating DNA.

To show that most of the DNA banding in the intermediate and light regions of CsCl-PI gradients was SV40 RI DNA and not breakdown of supercoiled viral DNA, DNA was extracted from the Triton supernatant and Triton nuclear fractions with phenol and analyzed on BND-

FIG. 2. Velocity sedimentation in CsCl gradients of SV40 DNA from Triton supernatant and Triton nuclear fractions. The Triton supernatant (A) and nuclear (B) fractions from cells labeled for 15 min, as well as the Triton supernatant (C) and Triton nuclear (D) fractions from cells labeled for 120 min were treated with SDS, mixed with ^{14}C -labeled polyoma DNA, and analyzed by velocity centrifugation in CsCl.

FIG. 3. Equilibrium centrifugation of SV40 DNA from Triton supernatant and Triton nuclear fractions in cesium chloride-propidium diiodide gradients. Samples were prepared by mixing SDS-treated ^3H -labeled SV40 fractions with ^{14}C -labeled polyoma DNA marker, propidium-diiodide, and CsCl . Solutions were adjusted to a final density of 1.515 g/cm^3 and centrifuged as previously described (8). Triton supernatant (A) and Triton nuclear (B) fractions from cells labeled for 15 min; Triton supernatant (C) and Triton nuclear (D) fractions from cells labeled for 120 min.

cellulose columns. Double-stranded DNA elutes from these columns at 1 M NaCl while replicating DNA remains bound but can be eluted by addition of caffeine (5, 14). Table 2 shows a comparison of the percent radioactivity which elutes from the two fractions of the BND-cellulose column with the percent radioactivity in the heavy (supercoiled) and intermediate plus light peaks from the CsCl -PI gradients. In all cases, there is a good correlation between the percentage of DNA in the intermediate and light regions of the CsCl -PI gradients and the percentage of DNA eluting as SV40 RI from the BND-cellulose column. We found in our experiments, in agreement with the observations of Levine and co-workers (14), that there is 10 to 20% reciprocal "spillover" of double-stranded and replicating DNA with these columns. This spillover could account for the differences shown in Table 2.

Buoyant densities of glutaraldehyde-fixed SV40 nucleoprotein complexes in CsCl . In the accompanying study (8), r-py complexes as well

as py complexes were shown to have the same ratio of protein to DNA as estimated from their buoyant densities after fixation with glutaraldehyde or formaldehyde. To obtain an estimate of the ratio of protein to DNA in the corresponding SV40 complexes, peak fractions of ^3H -labeled SV40 TSC and NEC from sucrose gradients were fixed in glutaraldehyde. Glutaraldehyde-fixed ^{14}C -labeled py complex was added as marker, and a portion of the solution was layered onto preformed CsCl density gradients and centrifuged to equilibrium (1). As shown in Fig. 4, SV40 TSC and NEC had buoyant densities identical to ^{14}C -labeled py complex. These results indicate that protein is bound to all forms of SV40 DNA at the same ratio of protein to DNA.

The average density reported for the glutaraldehyde-fixed polyoma nucleoprotein complexes was $1.437 \pm 0.008 \text{ g/cm}^3$, whereas the formaldehyde-fixed complexes had a slightly higher density, namely, $1.476 \pm 0.01 \text{ g/cm}^3$ (8). The reason for this density difference appears to be

TABLE 2. Fractionation of ^3H -labeled DNA from SV40 nucleoprotein complexes^a

Procedure	Fraction (%)			
	15-min pulse		120-min pulse	
	TSC	NEC	TSC	NEC
Propidium diiodide				
Heavy peak	40	13	73	65
Intermediate - light peak	60	87	27	35
BND-Cellulose				
1 M NaCl	57	27	87	71
1 M NaCl + 2% caffeine	43	73	13	29

^a Values represent percentage of counts recovered.

FIG. 4. Equilibrium centrifugation of glutaraldehyde-fixed polyoma and SV40 nucleoprotein complexes. SV40 Triton supernatant and Triton nuclear fractions were prepared from cells labeled for 15 min at 41.75 h after infection. SV40 TSC and NEC were isolated on 5 to 20% sucrose gradients. Peak fractions from each gradient were pooled and treated with glutaraldehyde. Fixed SV40 TSC (top) and SV40 NEC (bottom) were mixed with glutaraldehyde-fixed, ^{14}C -labeled polyoma TSC and centrifuged to equilibrium in preformed linear CsCl gradients.

related to the chemical effects of binding and not because the reagents are binding different amounts of protein.

As estimate of the ratio of protein to DNA in the complexes can be made by assuming that the relative density contributions of DNA and protein moieties are additive. SV40 DNA with a guanine plus cytosine content of 41% (6, 18) has a density in CsCl of 1.700 g/cm^3 . The origin of the protein component is not known, but since most proteins do not differ greatly in density, an approximate density estimate can be made by using an average value of the virion protein, namely, 1.295 g/cm^3 (3, 16). Using these density values, the ratio of protein to DNA of the glutaraldehyde-fixed complexes would be about 1.85 (65/35%) and, of the formaldehyde-fixed complexes, 1.24 (55/45%). Assuming that SV40 complex contains one supercoiled DNA molecule of about 3×10^6 daltons and only protein, SV40 complex would have a molecular weight in the range between about 6 and 9 million. Since the protein-to-DNA ratio in the complexes containing replicating DNA is the same, these complexes would range in molecular weight from that of SV40 complex to about twice that value.

Sedimentation of nucleoprotein complexes in sucrose gradients containing labeled ribosomes. When polyoma or SV40 DNA were used as markers, the apparent sedimentation rates of the nucleoprotein complexes varied with the type of gradient used and with the time of sedimentation. Furthermore, viral DNA appeared to sediment faster if Triton was not included in the gradients. Basically, viral DNA is not a suitable marker for the DNA-protein complexes because of the large differences in sedimentation rates and densities. Since ribosomal subunits are nucleoproteins with similar sedimentation coefficients and densities (2, 11, 20-22), nucleoprotein complexes were prepared and sedimented together with labeled ribosomal subunits used as markers.

The sedimentation pattern of ^3H -labeled py complex and ^{14}C -labeled ribosomal subunits is shown in Fig. 5. In this gradient, py complex appears as a single peak sedimenting ahead of the large ribosomal subunit. In buffers containing relatively high concentrations of EDTA and salt, the large ribosomal subunit is reported to have a sedimentation coefficient ($s_{20,w}^0$) of 47 (20, 21). Assuming linearity over short distances in the gradients, the sedimentation coefficient of py complex is about 56S.

Green and co-workers (9, 10) reported a sedimentation coefficient of 55S for the polyoma DNA-protein complex containing super-

coiled polyoma DNA, whereas White and Eason (23) reported a sedimentation coefficient of 44S for the corresponding SV40 DNA-protein complex. In both cases, these values were apparently derived from sedimentation in sucrose using polyoma 20S DNA or SV40 21S DNA as markers. As shown in Fig. 1, when the SV40 and the polyoma complexes were sedimented together in the same gradient, the SV40 complexes sedimented ahead of the polyoma complexes. Based on 56S as the sedimentation rate of py complex, the SV40 complex containing supercoiled DNA (SV40 complex) would have a sedimentation coefficient of about 61S, whereas SV40 complexes containing primarily 25S replicating DNA (r-SV40 complex) would sediment as broad heterogeneous peaks of about 70S (see Fig. 1C).

DISCUSSION

There are striking similarities between SV40 nucleoproteins and polyoma nucleoproteins extracted from infected cells by Triton lysis. Like polyoma, short-lived SV40 complexes containing replicating viral DNA (r-SV40 complexes) are not as easily extractable as complexes containing SV40 supercoiled DNA (SV40 complexes), suggesting that r-SV40 complexes are bound differently to nuclear sites. Furthermore, protein is bound to both replicating as well as supercoiled SV40 DNA at about the same ratio of protein to DNA as is found in polyoma nucleoproteins.

Fast sedimenting labeled complexes containing predominantly SV40 RI DNA were extracted after short pulses with ^3H -thymidine, while mostly slower sedimenting complexes containing SV40 supercoiled DNA were extracted after long pulses. Similar results were reported by White and Eason (23). In addition, we found some slow sedimenting complexes after short pulses containing a mixture of SV40 RI and SV40 supercoiled DNA. Thus, SV40 nucleoprotein complexes, like polyoma complexes, appear to sediment at rates proportional to the sedimentation rates of the DNA in the complexes rather than on the basis of whether the DNA is replicating or supercoiled. Since protein is bound to all forms of SV40 DNA to the same extent, the correlation between sedimentation rates of complexes and DNA indicate that the relative configurations of various forms of SV40 DNA are similarly affected by the addition of protein.

Considering that the sedimentation rates of SV40 complexes were reported to be about 20% less than those of polyoma (10, 23), a somewhat surprising finding was that SV40 complexes

FIG. 5. Velocity centrifugation of polyoma nucleoprotein complex and mouse ribosomal subunits. Cells were labeled with ^{14}C -uridine ($0.1 \mu\text{Ci/ml}$) from 8 to 26 h after infection. At 26 h ^{14}C -uridine was removed and the cells labeled for 2 h with ^3H -thymidine ($10 \mu\text{Ci/ml}$). Cells were lysed with Triton, and the supernatant fluid was analyzed on 5 to 20% sucrose gradients.

actually sedimented slightly faster than the corresponding polyoma complexes in our gradients. This discrepancy may be attributed to the use of viral DNA as markers in the previous studies. We found viral DNA unsatisfactory as a marker for several reasons. First, under sedimentation conditions which gave good resolution of the nucleoprotein complexes, viral DNA only sedimented about 20% into the gradient. Furthermore, when the composition of the gradients were varied, the relative sedimentation rates changed dramatically. Even the presence of Triton in the gradients affected the relative sedimentation rates in an unpredictable manner. These difficulties are no doubt related to the fact that DNA and nucleoproteins have different partial specific volumes and therefore the buoyancy forces in sedimentation will be different depending on the density and composition of the solvent solution.

To circumvent these difficulties ribosomal subunits were employed as markers since they have sedimentation rates and densities comparable to those of the SV40 and polyoma nucleoproteins (2, 11, 20). The S values of the complexes were calculated from the $s_{20,w}^0$ value reported for the large ribosomal subunit determined under conditions similar to those used in our studies, namely 47S (20). Nonetheless, values from 44 to 50S have been reported for large subunits in EDTA (2, 11). For these reasons, the actual S for py complex may be a value between 53 and 59S. It should be noted that, as reported (20), ribosomal subunits were

not stable in EDTA and were used within 72 h after being isolated.

In the preceding publication analysis of the sedimentation and buoyancy data revealed that polyoma nucleoproteins have somewhat more compact structures than would be expected for complexes with the DNA in configurations characteristic of free DNA (8). Analogous arguments would apply to SV40 complexes having sedimentation rates and densities like those of polyoma complexes. Previous data concerning the effects of chemicals and enzymes on SV40 complexes (23) suggest even further similarities in stability and chemical composition to the polyoma complexes. Of great interest will be a comparison of nucleoprotein complexes isolated after infection by "late" mutants from these two viruses.

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