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Isolation and Partial Characterization of Different Defective DNA Molecules Derived from Polyoma Virus

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Supercoiled DNA molecules purified from mouse cells infected with high-multiplicity-passaged polyoma virus has a broader size distribution and sediments more slowly than DNA derived from low-multiplicity-passaged virus. The shorter DNA molecules are predominately noninfectious. Virus populations containing distinct size classes of defective virus DNA were isolated by growing virus from single cells infected by a defective and nondefective helper virus (infectious center). This technique probably results in the cloning of defective virus particles. By applying the infectious center method to DNA from various fractions of sucrose gradients it has been possible to obtain shorter circular DNA molecules ranging in size from 50 to 95% of the unit-length polyoma DNA molecule. The shorter molecules in any one preparation are homogeneous in size. This class size is retained upon repeated passage of crude viral lysates at high multiplicity. Thus far, all the purified shorter DNA molecules tested appear to be noninfectious and largely resistant to cleavage by the R_I restriction enzyme. Some of the purified defective molecules have been found to interfere with the production of infectious virus upon co-infection with unit-length infectious polyoma DNA.

Heterogeneity in the size of both polyoma and SV-40 viral DNA extracted from virus passed at high-input multiplicities has been observed by a number of workers (1, 7-9, 11-13). Preparations of this type contain a significant number of shorter molecules that are predominately noninfectious (1, 9, 11-13). If these shorter molecules represent partially deleted virus molecules, they could prove extremely useful in the mapping and identification of viral genes, especially those involved in neoplastic transformation. The following report describes the isolation and partial characterization of different polyoma virus preparations greatly enriched in shorter supercoiled DNA molecules that range in size from 50 to 95% of the unit-length molecule.

MATERIALS AND METHODS

Virus stocks. The wild-type large plaque (PY) strain and the temperature-sensitive mutant TS-A have been described elsewhere (3). Virus stocks were grown on mouse embryo cells, and the virus was liberated from the infected cells by freeze-thawing or sonication in the presence of the growth media (4), or both.

Some of the properties of the high-multiplicity, low-multiplicity, and starting virus (medium multiplicity) are summarized in Table 1.

The initial starting virus used to produce the high-multiplicity virus had been passed with no great care over a number of years. The virus was passed undiluted for a number of passages, usually infecting 5×10^6 mouse embryo cells with 0.5 ml of lysate. High-multiplicity virus is denoted by DEL followed by the passage number. For instance, the fifth passage

TABLE 1. *Characteristics of virus produced at different multiplicities*

Characteristic	Low-multiplicity virus	Medium-multiplicity virus	High-multiplicity virus
PFU/HAU	10^6	10^5	As low as 10^3
PFU/physical particle	1/20	1/200	As low as 1/20,000
DNA PFU/μg of DNA	10^6	10^5	As low as 10^3
Yield of PFU/2 $\times$ 10^6 cells[a] (Single cycle)	8×10^9	8×10^8	As low as 8×10^6
Yield of DNA/5 $\times$ 10^6 cells[a]	5-8 μg	2-4 μg	As low as 0.5-1 μg

[a] Input multiplicity of 5 PFU/cell.

of the wild-type virus (PY) is referred to as PY-DEL-5 and the sixth passage of TS-A as TS-A-DEL-6.

Low-multiplicity virus was derived from the medium-multiplicity virus by isolating single plaques from dishes containing less than four plaques per dish. The plaques were picked into 1 to 1.5 ml of growth medium and freeze-thawed three times. Each plaque usually contained between 10^4 and 10^6 PFU. Approximately one half of the virus from the plaque was used to infect 5×10^6 cells. The virus from this infection was harvested after 5 to 7 days at 37 C or 10 to 14 days at 31.5 C. ^{14}C-labeled viral DNA purified from cells infected with low-multiplicity virus at an input of 1 PFU/cell was used as the standard unit-length marker DNA in sucrose gradients.

Assay of virus and infectious DNA. The plaque assay used to titer infectious virus and the haemagglutination assay used to titer physical particles have been described previously (4). Infectious viral DNA was assayed on mouse embryo cells by the DEAE-dextran method of Thorne et al. (9).

Infectious center plaques. The cloning of individual defective virus particles from high-multiplicity-passaged virus was attempted by growing virus from single cells infected by a defective and a nondefective particle. Mouse embryo cells were infected at a multiplicity of less than, or equal to, 1 PFU/cell, but at a multiplicity of greater than one particle per cell. The input multiplicity depended on the defectiveness of the stock as estimated from the PFU/hemagglutination unit (HAU) ratio. Usually a variety of input multiplicities were used for each virus preparation.

After virus absorption the cells were washed three times with medium and then incubated for 2 to 3 h in the presence of receptor-destroying enzyme and anti-polyoma antiserum to reduce and inactivate the unabsorbed virus (4). The infected cells were then trypsinized and reseeded at concentrations ranging from 50 to 5,000 cells with 2×10^6 uninfected mouse embryo cells per 50-mm petri dish. After 2 to 3 h when the cells had settled, the media were removed and agar was added as for a normal plaque assay.

When the infectious center plaques developed they were picked into 1 to 1.5 ml of medium with a wide-bore pipette. The picked plaques were then freeze-thawed three times to release the virus from the plug of agar. The virus was then grown up on mouse embryo cells at high multiplicity. The supercoiled DNA derived from virus produced by these infectious center plaques was then sedimented in neutral sucrose gradients.

Viral DNA extraction and purification of supercoiled molecules. Infected mouse embryo cells were incubated for either 4 days at 37 C or 7 to 8 days at 32 C (usually in the presence of 2 μCi of [^{3}H]thymidine and 1 μg of cold thymidine of medium per milliliter) before viral DNA was extracted by the selective method of Hirt (5). The Hirt supernatant fluid was extracted with phenol, and the nucleic acid was precipitated by the addition of 2 volumes of ethanol at −20 C. The supercoiled and nonsupercoiled DNA were separated by using an ethidium bromide-cesium chloride equilibrium gradient (6).

Neutral sucrose gradients. Linear 5 to 20% (wt/vol) sucrose density gradients containing 0.14 M NaCl, 0.01 M Tris-hydrochloride (pH 8.5), and 0.004 M EDTA were used for sedimentation analysis of purified supercoiled DNA. [^{3}H]thymidine-labeled DNA samples with or without [^{14}C]thymidine-labeled marker DNA in volumes of 0.2 to 0.3 ml were layered on top of gradients that were centrifuged in an SW40 rotor at 28,000 rpm for 17 h at 19 C. The tubes were pierced from the bottom, and either 5 or 10 drop fractions were collected on 1^2-inch (2.54^2 cm) Whatman no. 1 filter paper. There were 64 to 67 10-drop fractions or 128 to 134 5-drop fractions. The DNA was acid precipitated and the radioactivity determined. The size of slower sedimenting peaks, expressed as a percentage of the unit-length marker DNA, was calculated from the equation of Vinograd (2).

Cleavage with R_1 endonuclease. The R_1 endonuclease was a gift of W. R. Folk. The digestion was carried out in a buffer containing 0.05 M Tris-chloride, 0.05 M NaCl, and 0.01 M $MgCl_2$ at 37 C for 30 min. The enzyme was in 20-fold excess at a ratio of 1 μliter of enzyme to 1 μg of DNA. The reaction was stopped by the addition of EDTA to a final concentration of 0.03 M.

RESULTS

Isolation of TS-A-shorter DNA molecules. It was thought that a TS-A viral DNA deleted in genes coding for capsid proteins could be rescued by a temperature shift of mouse cells transformed and maintained at the nonpermissive temperature (10). Therefore, the isolation of shorter DNA molecules from TS-A was attempted.

Upon repeated passage of TS-A virus at high-input multiplicity, it was observed that the yield of particles as well as the yield of plaque-forming units decreased markedly. At the sixth high-multiplicity passage the PGU-HAU ratio was reduced to 2×10^3, which was 50-fold less than the starting virus inoculum and 500-fold less than low-multiplicity-produced virus. This is equivalent to about 1 PFU/10,000 physical particles (usually about 50% of these particles do not contain any viral DNA). The major part of the supercoiled DNA extracted from TS-A-DEL-6-infected cells sedimented more slowly than low-multiplicity marker unit-length DNA in neutral sucrose gradients. A comparison of the widths of the two sedimenting bands (Fig. 1) indicates that the TS-A-DEL-6 DNA is more heterogenous in size than the low-multiplicity marker DNA.

To see if complementation to form a plaque between different defective virus particles present in the TS-A-DEL-6 virus population could be detected, 43 infectious center plaques were isolated after infection with TS-A-DEL-6 virus at a multiplicity of 0.01 PFU/cell (approximately 100 physical particles/cell). All 43 infec-

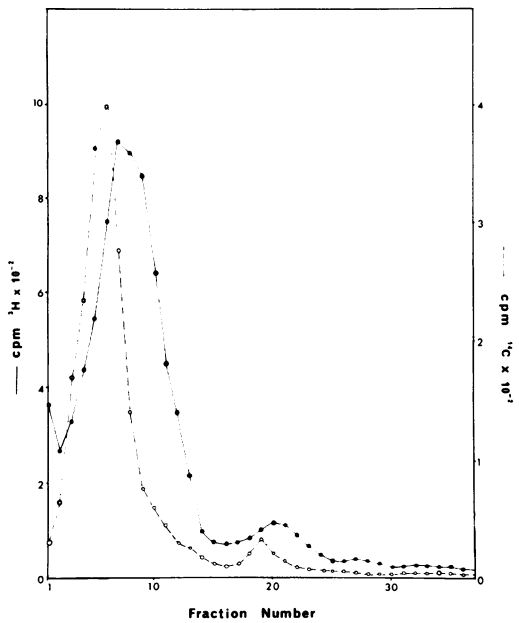

FIG. 1. *Neutral sucrose gradient sedimentation analysis of TS-A-DEL-6 DNA. Symbols:* ●, *^{3}H-labeled TS-A-DEL-6 DNA;* ○, *^{14}C-labeled unit-length marker DNA. In this and the following figures only data from the lower half of the sucrose gradients are presented.*

tious center plaques when replaqued showed linear plaque formation with dilution. This suggests that these plaques were not produced by complementation of two or more defective virus particles. All the plaques tested contained between 10^4 and 10^6 PFU.

To isolate shorter DNA molecules, seven infectious center plaques derived from an infection of TS-A-DEL-6 at an input multiplicity of 0.001 PFU/cell (approximately 10 physical particles/cell) were grown up as described in Material and Methods. The resulting virus was used to infect a large number of mouse embryo cells from which supercoiled DNA was extracted and purified.

The DNA from each of the seven plaques was sedimented in a neutral sucrose gradient in the presence of a marker from low-multiplicity viral DNA. The width of each of the seven DNA-sedimenting bands indicated that they were more homogeneous in size than the DNA from the uncloned TS-A-DEL-6 DNA. DNA from three of the plaques sedimented coincident with the marker, whereas two sedimented with characteristics of molecules 5 to 10% shorter and two approximately 15% shorter.

The infectivity of the fractionated DNA of one of the plaques (TS-A-DEL-6-2) containing 15% shorter molecules was assayed (Fig. 2). It was found that the peak of infectivity sedimented faster than the bulk of the DNA, and in the same region as the low-multiplicity marker DNA. In the fractions containing most of the 15% shorter DNA, only background infectivity was present. Eighty-seven plaques resulting from infection with DNA from the light side of the infectivity peak in the region of the bulk of the DNA were picked. All of these plaques contained 10^4 to 10^6 PFU and, on replaquing, produced a linear dose response with dilution.

Clonal isolation of PY shorter DNA molecule. Because the shorter molecules derived from TS-A appeared to carry little or no infectivity, complementation between wild-type shorter DNA molecules and the various temperature-sensitive mutants, including TS-A, might be feasible. Therefore, the isolation of shorter DNA populations was attempted from wild-type virus. After the second passage at high multiplicity of wild-type virus, infectious center plaques were isolated and grown up. Two out of three plaques tested showed a

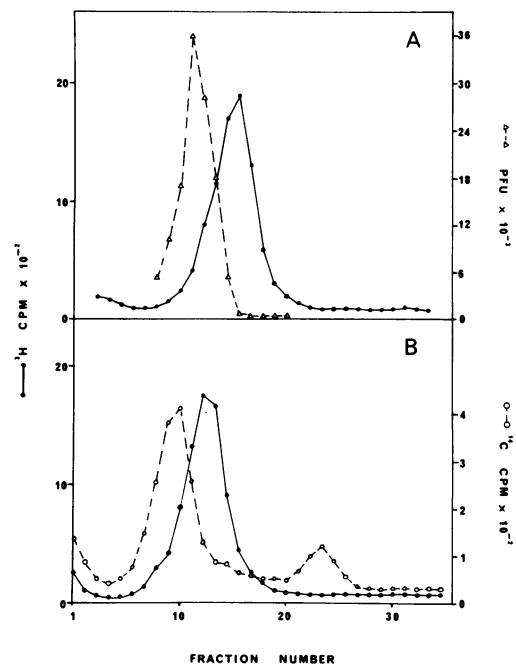

FIG. 2. *Neutral sucrose gradient sedimentation analysis of DNA from infectious center plaque TS-A-DEL-6-2. (A) DNA infectivity of various gradient fractions of TS-A-DEL-6-2 DNA. Symbols:* ●, *^{3}H-labeled TS-A-DEL-6-2 DNA;* △, *infectious DNA plaque-forming units. (B) Sedimentation behavior of TS-A-DEL-6-2 DNA in relation to unit-length marker DNA. Symbols:* ●, *^{3}H-labeled TS-A-DEL-6-2 DNA;* ○, *^{14}C-labeled unit-length marker DNA.*

sharp single peak of DNA about 5 to 10% shorter than the marker, whereas the DNA from the third plaque co-sedimented with the marker.

An attempt was made to enhance for even shorter populations by growing up virus from the DNA from the light side of high-multiplicity DNA peaks. Supercoiled DNA, purified from PY-DEL-5 sedimented through a neutral sucrose gradient, showed a broad distribution and sedimented more slowly than the marker DNA (Fig. 3). DNA from the peak fraction and a number of fractions from the light side of the peak of the PY-DEL-5 DNA were used to infect mouse cells. The viruses produced from these DNA infections were used to infect a large number of mouse cells from which the supercoiled DNA was purified and analyzed.

Figure 4 shows the sedimentation characteristics of DNA derived from these various fractions. It can be seen that the DNA obtained from the peak fraction 14 is similar in its size distribution to that of the parental PY-DEL-5 gradient (Fig. 3A). With the DNA derived from the lighter fractions, two peaks of DNA were resolved; one co-sedimented with the marker DNA, whereas the other sedimented more slowly depending on its origin in the parental gradient. It was found that the amount of slower sedimenting DNA decreases much more quickly than the unit-length DNA upon infection with increasing dilutions of the virus derived from the various fractions. Thus it would appear that

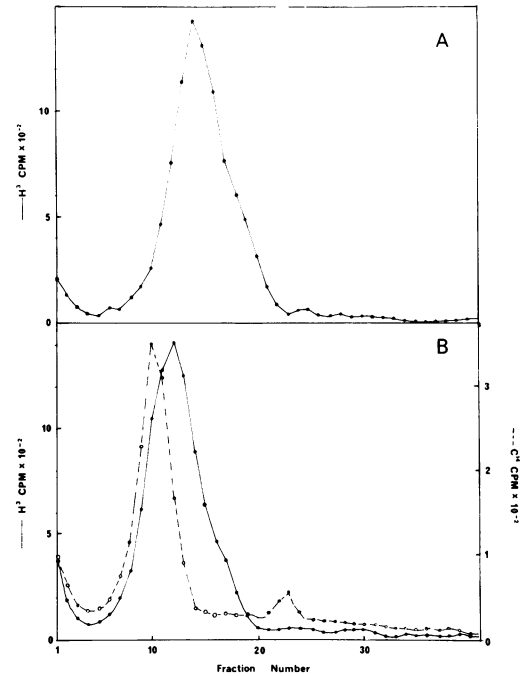

FIG. 3. *Neutral sucrose gradient sedimentation analysis of PY-DEL-5 DNA. (A) Sedimentation of PY-DEL-5 DNA. The DNA of fractions 14–22 was used to make virus stocks that were further analyzed (see Fig. 4). (B) Sedimentation behavior of PY-DEL-5 in relation to unit-length marker DNA. Symbols: ●, ^{3}H-labeled PY-DEL-5 DNA; ○, ^{14}C-labeled unit-length marker DNA.*

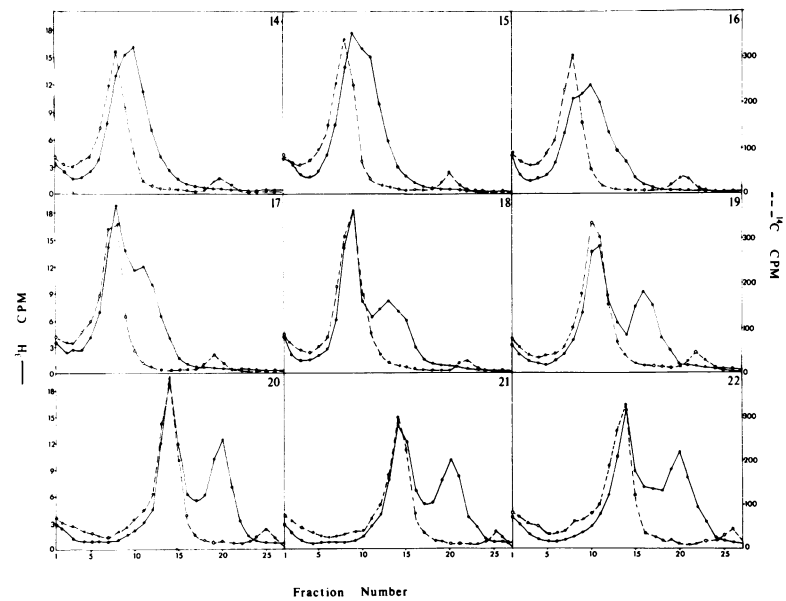

FIG. 4. *Neutral sucrose gradient sedimentation analysis of supercoiled DNA isolated from mouse cells infected with virus derived from the DNA of gradient fractions 14–22 (Fig. 3A). Symbols: ●, ^{3}H-labeled DNA derived from gradient fractions 14–22; ○, ^{14}C-labeled unit-length marker DNA.*

the shorter molecules are defective and are dependent on the unit-length molecules for their growth.

The viruses derived from fractions 21 and 22 (Fig. 4) were used to produce infectious center plaques. Fourteen infectious center plaques were grown up and their DNA was analyzed. In all cases most of the DNA co-sedimented with the marker. In most of the preparations a shoulder or another peak of DNA was distinguishable. Table 2 lists the approximate size of the slower sedimenting DNA relative to the unit-length molecule. Figures 8A and 9A show representative gradients of preparations containing molecules 75 and 60% of the unit-length molecule, respectively.

Infectivity of the shorter molecule. It was already found that the infectious DNA derived from TS-A-DEL-6-2 (Fig. 2) sedimented in the region of the unit-length molecule rather than with the bulk of the DNA. It is of interest to know if all the DNA sequences present in the unit-length molecules of polyoma virus are essential for the production of new infectious virus. Thus it was decided to look at the infectivity of other isolated shorter DNA molecule populations.

The 75% DNA molecules derived from virus of the plaque designated 21-2 were purified through two sucrose gradients. The final DNA preparation (Fig. 5) contained 3×10^2 PFU/μg of DNA. This is equivalent to a 10^4-fold decrease in terms of plaque-forming units per molecules of DNA in comparison with low-multiplicity viral DNA. (Table 1). Six plaques that arose from the 21-2 75% DNA were picked and replaqued. Individual plaques were grown up at low multiplicity. Supercoiled DNA obtained from the virus from these plaques was analyzed in sucrose gradients and each plaque that derived DNA showed a single peak that co-sedimented with the marker DNA, as illustrated in Fig. 6. This result suggests the small amount of infectivity present in the 75% molecules of plaques 21-2 is probably due to contamination with unit-length molecules.

TABLE 2. *Sizes of shorter DNA molecules of virus derived from fraction 21 and 22*

Infectious center plaques	Approximate size of slower sedimenting DNA[a]
Fraction 21	
21-1	75%
21-2	75%
21-3	50%
21-4	50%
21-5	75%
21-6	None
21-7	None
Fraction 22	
22-1	60%
22-2	None
22-3	75%
22-4	None
22-5	90%
22-6	75%
22-7	65%

[a] The sizes of the slower sedimenting DNA, expressed as a percentage of the unit-length marker DNA, was calculated from the equation of Vinograd (2).

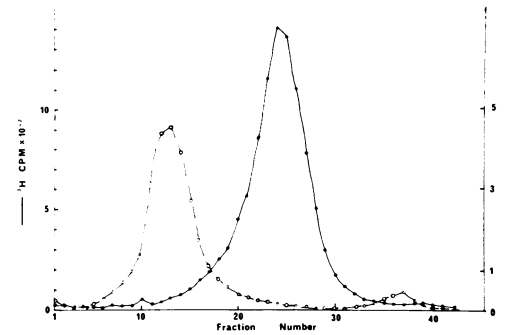

FIG. 5. *Neutral sucrose gradient sedimentation analysis of 75% DNA derived from virus from infectious center plaque 21-2. The peak continuing the 75% DNA was isolated from two successive neutral sucrose gradients before being sedimented with the marker DNA. Symbols: ●, ^{3}H-labeled 75% 21-2 DNA; ○, ^{14}C-labeled unit-length marker DNA.*

The infectivity found in 90 and 50% molecules, isolated after treatment with the restriction enzyme R_I (see below), was less than 15 and 125 PFU/μg of DNA, respectively. All the shorter molecules so far analyzed are defective in the production of new infectious virus.

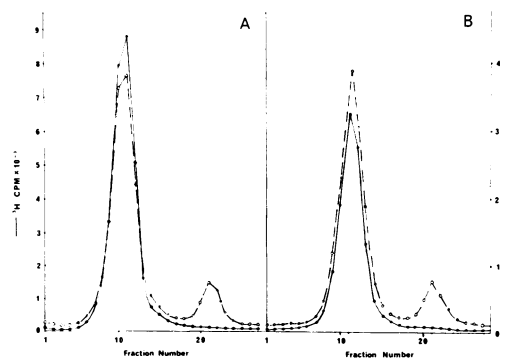

FIG. 6. *Neutral sucrose gradient sedimentation analysis of DNA derived from virus from two plaques that arose after infection with purified 75% 21-2 DNA (Fig. 5). Symbols ●, ^{3}H-labeled DNA from 75% 21-2 plaques; ○, ^{14}C-labeled unit-length marker DNA.*

Restriction enzyme R_I sensitivity of the shorter molecule. Circular polyoma virus DNA is cleaved to linear molecules at one unique region by R_I restriction enzyme (D. Robberson and M. Fried, manuscript in preparation). Viral DNA derived from plaques grown at low multiplicity was found to be greater than 95% sensitive to cleavage to linear molecules with the R_I restriction enzyme (Fig. 7).

A number of preparations containing both a peak of unit-length molecules and a peak of shorter molecules were tested for their sensitivity to cleavage with R_I. So far none of the shorter molecules (14 isolates) were found, to any great degree, to be sensitive to cleavage with the R_I enzyme. Unit-length molecules in the same preparations were cleaved to linear molecules (Fig. 8 and 9). If detectable amounts of the shorter molecules were sensitive to cleavage with R_I, a disappearance of the shorter supercoiled molecules should have occurred and a peak of DNA sedimenting at the region where the shorter linear molecules sediment would have been observed.

Treatment with R_I revealed that resistant supercoiled molecules about 85 to 95% of the unit-length molecules are present in varying amounts in most preparations produced at high multiplicity. These R_I-resistant molecules are particularly obvious in preparations containing DNA less than 80% where they appear as a shoulder or a separate peak (Fig. 8 and 9).

Treatment with R_I was used to purify the shorter molecules. The preparations were treated with R_I, and the resistant shorter supercoiled molecules were separated from the sensitive linear molecules by equilibrium density centrifugation. Velocity sedimentation was used as a further purification step for those molecules of 80% or less.

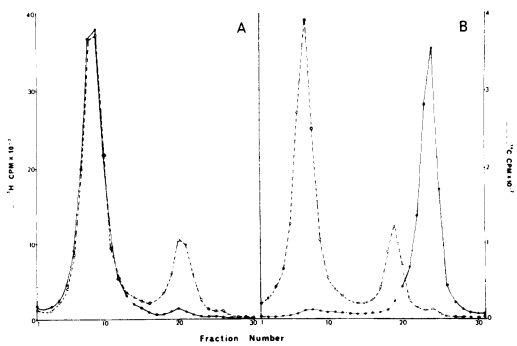

FIG. 7. *Neutral sucrose gradient sedimentation analysis of low-multiplicity DNA before and after treatment with restriction enzyme R_I. (A) Before treatment with R_I. (B) After treatment with R_I. The marker DNA was added after the R_I reaction had been terminated. Symbols: ●, ^3H-labeled low-multiplicity DNA; ○, ^{14}C unit-length marker DNA.*

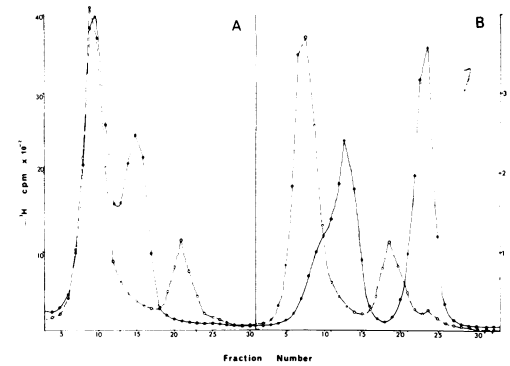

FIG. 8. *Neutral sucrose gradient sedimentation analysis of DNA from infectious center plaque 22-3 (Table 2) before and after treatment with restriction enzyme R_I. (A) Before treatment with R_I. (B) After treatment with R_I. The marker DNA was added after the R_I reaction had been terminated. Symbols: ●, ^3H-labeled 22-3 DNA; ○, ^{14}C-labeled unit-length marker DNA.*

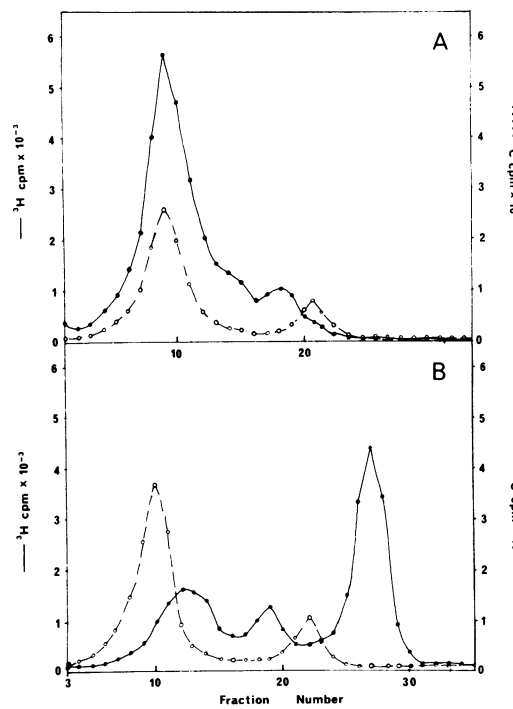

FIG. 9. *Neutral sucrose gradient sedimentation analysis of DNA from infectious center plaque 22-1 (Table 2) before and after treatment with restriction enzyme R_I. (A) Before treatment with R_I. (B) After treatment with R_I. The marker DNA was added after the R_I reaction had been terminated. Symbols: ●, ^3H-labeled 22-1 DNA; ○, ^{14}C-labeled unit-length marker DNA.*

Interference. Experiments were performed to determine the effect of the various shorter molecules on the growth of low-multiplicity-produced wild-type DNA. Mouse cells were infected with different preparations of purified shorter DNA plus wild-type low-multiplicity DNA. The yields of virus produced at 31.5 and 38.5 C of the dual infections were compared with the yield produced by the wild-type DNA alone. Table 3 lists the results of such an experiment. Whereas co-infection with circular supercoiled SV-40 DNA, or linear DNA from uninfected cells, produced little or no inhibition of the production of wild-type virus, some of the preparations of shorter DNA produced as much as a 10-fold inhibition. Those shorter molecules derived from TS-A virus are equally effective in inhibiting at the permissive and nonpermissive temperature. This inhibitory effect will present a problem in the use of genetic complementation tests to identify viral genes present in some of the shorter DNA molecule preparations.

DISCUSSION

It has been possible by the techniques described above to produce different viral DNA preparations that are greatly enriched in individual distinct classes of supercoiled, defective DNA molecules, ranging in size from 50 to 95% of the unit-length polyoma DNA molecule. The rationale of the infectious center method was to clone defective virus particles by growing the progeny of individual cells infected by only one defective particle in the presence of a nondefective helper virus particle. The observation that 30 to 60% of the infectious center derived virus isolates contained no detectable shorter molecules suggests that cells that produced shorter molecules were infected by only one (or just a few) defective particles. The success of this method in the cloning of individual particles is supported by the following observations. (i) The shorter molecules in any one isolate are homogeneous in size and this size molecule is retained upon repeated passage of crude viral lysates at high input multiplicity. (ii) Some of the purified shorter molecules have been shown to be homogenous with respect to their nucleic acid sequences by heteroduplex studies (Robberson and Fried, submitted for publication) and by the production of molar equivalents of fragments after treatment with multicut restriction enzymes (Griffin and Fried, manuscript in preparation). (iii) The shorter molecules are representative of the size distribution of the DNA population of the virus used to produce the infectious centers (Fig. 4 and Table 2).

TABLE 3. *Interference of different DNAs with PY DNA*[a]

DNA	Yield of PFU/culture at 31.5 C	Yield of PFU/culture at 38.5 C
PY + 85% TS-A-DEL-6-2	1.3×10^6	2.5×10^5
PY + 85% TS-A-DEL-6-5	2.5×10^6	5.5×10^5
PY + 95% PY-DEL-2-3	6.0×10^6	9.5×10^5
PY + 75% 22-3	3.1×10^6	5.5×10^5
PY + SV − 40	1.3×10^7	2.5×10^6
PY + uninfected mouse cell linear DNA	1.6×10^7	3.5×10^6
PY	1.5×10^7	3.0×10^6

[a] A total of 8×10^5 mouse embryo cells were infected with 0.25 μg of unit-length (PY) DNA in the presence or absence of 0.05 μg of each DNA to be tested. After infection the cells were trysinized and 4×10^5 cells were plated into each of two 35-mm petri dishes with medium containing receptor-destroying enzyme (4). One half of the dishes were incubated at 38.5 C and the other half at 31.5 C. After 68 h at 38.5 C or 144 h at 31.5 C, the virus was harvested. The yield of plaque-forming units per culture is shown.

The finding that shorter molecules retain their size when grown with different isolates of unit-length infectious DNA molecules (Fried, unpublished data) suggests that the shorter molecules are actually derived from the original population as opposed to being continuously generated by particular unit-length molecules. The infectious center method requires only that the defective particle being isolated can coexist with the infectious unit-length molecule in the formation of a plaque and thus is nonselective to this extent.

In most high-multiplicity-passaged virus stocks, 85 to 95% length DNA makes up the majority of the non-unit length DNA molecules (see Fig. 1 and 3). This may explain the presence of 85 to 95% molecules, as a second size class, in some of the defective DNA isolates. This second size class was usually observed in isolates containing defective DNA of less than 80% unit length (Fig. 8 and 9). The defective DNA molecules so far studied have been noninfectious and have depended, for continued passage, on complementation with unit-length infectious viral DNA. The shorter DNA is thus itself maintained by passage of virus stocks at high multiplicity. This passage has probably led, in some cases, to the generation of the 85 to 95% shorter molecules discussed above. The prevalence of the 85 to 95% molecules may be explained by the less efficient encapsidation into a stable particle of smaller than 85% DNA, thus conferring a selective advantage to these newly generated larger defective molecules. It cannot be ruled out, however, that the second

class of defective DNA is due to infection by more than one defective particle of the cell that produced the infectious center.

So far all the shorter molecules tested have been found to be predominately noninfectious and resistant to cleavage with the R_I restriction enzyme. The reason for this resistance to cleavage by R_I is unclear at this time, but may be revealed when the structure of these molecules is determined. The loss of the R_I cleavage site might indicate that this region is not very essential for viral growth or that the defectives are generated by a common mechanism.

The finding that some of the shorter molecules interfere with the production of infectious virus is not too surprising. On continued high-multiplicity passage both the yields of infectious virus and virus particles decrease markedly. Continued high-multiplicity passage also results in the production of predominately shorter than unit-length supercoiled DNA. Perhaps this type of interference plays some role in vivo. Defective viruses, whether due to an addition of host sequences or repetitions of specific viral sequences, may have a selective advantage and replicate faster and at the expense of the infectious virus. Such a mechanism would lead to the slowing down of the virus infection within a high-multiplicity virus area, such as a lesion, and might allow more time for the host defense mechanism, like the immune response, to act before the virus infection has damaged too many cells.

The transforming ability of different purified defective DNA is now under investigation. By this means it may be possible to identify those portions of the viral genome necessary for neoplastic transformation, as well as other viral functions.

Some of the classes of molecules isolated contain only a portion of the viral genome (Robberson and Fried, manuscript in preparation). Thus, the isolation procedures described above present a way of obtaining large and probably homogeneous quantities of a restricted portion of this small viral genome that can be replicated in vivo. The biological, biochemical, and genetic studies of these deleted molecules should lead to a better understanding of the properties of polyoma virus.

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