Multiplication of Polyoma Virus in Mouse-Hamster Somatic Hybrids: a Hybrid Cell Line Which Produces Viral Particles Containing Predominantly Host Deoxyribonucleic Acid

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The multiplication of polyoma virus in a mouse-hamster (3T3 x BHK) somatic hybrid line (10A), which, although permissive for viral multiplication, produces very low amounts of virus, has been studied. In this cell line, the efficiency of productive infection is high, but the yield of infectious virus is on the order of 0.5% of that of 3T3 cells. The amount of viral deoxyribonucleic acid (DNA) synthesized by these cells upon infection is about 5% of that of 3T3 cells. An examination of the virus produced in hybrid 10A revealed that it was only one-tenth as infectious as the virus grown in 3T3. Although the viral DNA synthesized in the infected 10A cells is normal, the DNA extracted from purified virus grown in 10A consists of ~10% of normal, supercoiled polyoma DNA molecules and of ~90% linear DNA molecules with a sedimentation coefficient of 14 to 16S. These DNA molecules appear to be of cellular origin but contain a limited amount of viral DNA sequences. The host DNA-containing particles are not infectious but appear to possess some biological activity; they give rise to a weak complementation effect, and part of them are able to induce T-antigen synthesis. In addition, the host DNA present in these particles is predominantly that which has been synthesized after infection. The correlation between the block in viral DNA synthesis in this cell line and the abnormal encapsidation of host DNA is discussed.

Host cell factors may in many cases determine the outcome of the infection of a susceptible cell by a virus. A demonstration of the control exerted by the host cell on viral multiplication is given by the interaction that the small deoxyribonucleic acid (DNA) oncogenic viruses, polyoma and simian virus 40, exhibit with cells *in vitro*. These viruses in fact multiply only in some cell types, which are called permissive, whereas in others they perform only malignant transformation. This latter phenomenon is not accompanied by any significant viral multiplication or cell death. In the case of polyoma virus, mouse cells support almost exclusively a productive interaction (9); nearly all hamster cells, however, support only a transforming interaction (17). Permissiveness to polyoma multiplication appears to behave like a dominant character. Previous work (4) has, in fact, shown that somatic hybrids between 3T3 mouse cells and BHK hamster cells supported

viral multiplication, provided that they contained a diploid, or almost diploid, mouse chromosome complement. However, some of these hybrids produced very low amounts of virus. This phenomenon was due to a drastic reduction of the burst size, apparently caused by an impairment of viral DNA synthesis, whereas other viral functions did not seem to be affected. The most extreme in this behavior was hybrid line 10A. This hybrid was also notable in that it produced a viral yield which seemed to contain a high proportion of noninfectious particles.

In an effort to gain information on the regulation of viral functions caused by host cell factors, the characteristics of polyoma multiplication in this cell line have been studied in detail. The results presented in this paper show that when polyoma infects 10A cells, viral early functions appear to be fully expressed, but viral DNA synthesis is grossly impaired. In addition, at least 90% of the virus particles produced appear to contain predominantly cellular DNA in their

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virions. These particles are not infectious but possess some biological activity.

MATERIALS AND METHODS

Cells. The cells used were the 3T3 mouse line (18) and a BHK $\times$ 3T3 hybrid called 10A. The formation and isolation of this hybrid line have already been described (4).

Cell cultures were grown in Dulbecco's modification of Eagle's medium, supplemented with 10% calf serum. The 10A hybrid line, although chromosomally stable, was never serially propagated for longer than 30 to 45 days.

Virus. The small-plaque variant of polyoma virus (Toronto strain) was used in all of the experiments. Virus stocks were grown on 3T3 cells, and viral purification was performed essentially by the method of Winocour (23). Cells were subjected to ultrasonic disintegration, and the virus was eluted by making the medium slightly alkaline ($\sim$ pH 8.5) and incubating several hours at 37 C, in most cases in the presence of receptor-destroying enzyme (1 unit/ml). After centrifugation at $10,000 \times g$ to remove the cell debris, the virus was concentrated by high-speed centrifugation and subjected to two or three cycles of equilibrium density gradient centrifugation in CsCl (initial density 1.30). In some cases, the whole cell lysate was treated with sodium deoxycholate before spinning out the cell debris.

Viral infectivity was determined by plaque assay on monolayers of 3T3 cells (3). Hemagglutinating ability was titered by using guinea pig erythrocytes (2).

Viral infection. Cells in monolayer were infected with 0.2 ml of virus suspension per 60-mm petri dish or 0.5 ml per 100-mm petri dish. After 2 hr, the cells were washed with isotonic tris(hydroxymethyl)amino-methane (Tris) buffer to remove unadsorbed virus and incubated in medium containing 5% calf serum. Unless otherwise specified, infection was carried out by using growing cells.

Techniques have been described (4) for the determination of the frequency of cells synthesizing polyoma capsid antigen or polyoma T-antigen.

DNA extraction: from noninfected cells. Purified cell DNA was extracted by the method of Marmur (13), with minor modifications.

From infected cells. This was generally performed by the method of Hirt (11) or by sodium dodecyl sulfate (SDS)-phenol extraction (8). Purified supercoiled polyoma DNA (form I; reference 21) was also prepared from infected 3T3 cells; in this case, Hirt extracts of infected cells were shaken once with phenol, and the DNA was precipitated with ethanol and subjected to one or two cycles of dye buoyant density centrifugation in ethidium bromide, CsCl gradients (16).

From virus. Viral DNA was extracted from purified polyoma virus by SDS treatment (0.6%, 1 hr at 37 C) followed by NaCl precipitation of most of the SDS-protein complex. In most cases, this was followed by one phenol extraction, and the DNA preparation was then dialyzed extensively. Direct phenol extraction (22) was also used in some cases.

DNA infectivity. DNA infectivity was measured by the method of Pagano et al. (15) by infecting monolayers of 3T3 cells in the presence of diethylaminoethyl (DEAE) dextran.

DNA-DNA hybridization. DNA-DNA hybridization was performed as described by Aloni et al. (1) with the following modification. The DNA-containing filters were preincubated in $3 \times$ SSC (0.45 M NaCl plus 0.045 M sodium citrate) containing 0.03% bovine serum albumin (BSA) for 6 hr at 60 C. After preincubation, the filters were drained and placed in a vial containing $3 \times$ SSC (no BSA) and the labeled, sonically treated DNA to be tested. Incubation was then continued for about 20 hr.

Density gradients and counting of radioactivity. The density gradient techniques and methods of counting radioactivity have been previously described (4).

RESULTS

Multiplication of polyoma virus in hybrid 10A. When 10A cells were infected with polyoma virus at multiplicity of infection (MOI) of $\sim$ 100 plaque-forming units (PFU)/cell and the results compared with those obtained in the parental 3T3 cells, it could be seen (Table 1) that in both lines approximately all of the cells synthesized polyoma T antigen and comparable frequencies of cells produced virus. The yield, however, was markedly different. 10A cells produced less than 1% of the amount of infectious virus produced by 3T3. Since the frequency of virus-producing cells was similar, it was apparent that the reduction of yield was due to a reduction of the burst size, which in several different experiments averaged about 0.5% of that of 3T3. Kinetics of virus production were slightly slower in 10A than in 3T3 cells. Both cell types were killed as a result of viral infection.

Viral DNA synthesis in infected 10A cells was then studied (Fig. 1). The incorporation of radioactive thymidine into polyoma DNA in 10A cells

TABLE 1. Infection of 10A and 3T3 cells with polyoma virus^a (one-step growth data)

Cells	Frequency of cells synthesizing T antigen (%) ^b	Frequency of virus producing cells (%)		Yield (PFU/10 ⁶ cells)	Burst size (PFU/cell)
		V-antigen positive cells ^c	Infectious centers		
3T3	90-100	57	60	1.8×10^9	3,000
10A	90-100	34	39	1.1×10^7	27

^a Multiplicity of infection = 100 plaque-forming units (PFU)/cell.

^b Determined at 24 hr after infection by immunofluorescence.

^c Determined at 31 hr after infection by immunofluorescence.

FIG. 1. Viral DNA synthesis in hybrid 10A. 10A (○) and 3T3 (●) cells were infected with polyoma virus (100 PFU/cell) and exposed to ³H-thymidine (4 μCi/ml; 1.7Ci/mmol) for the periods indicated. The cultures were washed in isotonic Tris buffer, and the viral DNA was extracted by the method of Hirt (11). A sample of the extract was layered on 3.8 ml of CsCl (density 1.50, pH 8.0) and centrifuged for 4 hr at 35,000 rev/min in the Spinco SW50 rotor at 21°. The ordinate gives the counts/min (trichloroacetic acid-insoluble) in the DNA of each fraction. The inset shows the value for 10A on an expanded scale. The peaks of radioactivity are all in the 20S region.

was drastically reduced with respect to 3T3 cells. A slight delay in the appearance of viral structural proteins in the nuclei of infected cells was also observed (Fig. 2). On the average, the total amount of viral DNA synthesized by an infected 10A cell before lysis was 5% of that of 3T3. This small amount of viral DNA synthesis was required

for the synthesis of viral structural proteins since inhibition of DNA synthesis by cytosine arabinoside completely inhibited the appearance of viral capsid antigen in the nuclei of infected cells (4).

The viral DNA synthesized in 10A cells was found to be normal with respect to size, buoyant density in ethidium bromide-CsCl gradients, and

infectivity. Table 2 shows the results of an experiment in which 10A or 3T3 cells were labeled from 15 to 31 hr after infection, and the viral DNA was extracted and purified by band centrifugation (20). It can be seen that the ratio of infectivity to radioactivity incorporated is substantially the same for both cell types. Table 2 shows also that, when the yield of infectious virus and the amount

of viral DNA synthesized in infected 10A cells are compared, the reduction in viral DNA synthesis still does not seem sufficient to account for the reduction of the infectious yield. However, pulse-chase experiments showed the viral DNA to be as stable in 10A cells as in 3T3.

It was considered of interest to determine whether other viral functions were affected in 10A cells. Infection of confluent, resting 10A cells with polyoma virus led to induction of cellular DNA synthesis (8). The extent of stimulation appeared to be about the same as in 3T3 (3). As expected, under those conditions viral DNA represented a very small fraction of the DNA synthesized upon infection (~1% as opposed to 20 to 25% for 3T3). Breakdown of cell DNA (5) occurred as in mouse cells, both after infection of growing or of resting cells.

Characteristics of polyoma virus produced in 10A cells. As stated before, the reduction of infectious virus yield in 10A cells did not seem to be completely accounted for even considering the low rate of viral DNA synthesis. This observation seemed to find some explanation in the discovery that the yield of polyoma particles produced in 10A cells was largely noninfectious. This was first suggested by the relatively high (with respect to infectivity) hemagglutination (HA) titer of the crude virus lysates (Table 3). Examination of the purified virus confirmed this finding. The PFU/HA ratio (7), which is on the order of 10^5 for virus grown in 3T3 cells, was 10^4 or less for the 10A-grown virus. This suggested that the virus produced in 10A (hereafter referred to as polyoma 10A) was one-tenth as infectious as polyoma grown in 3T3. Since the PFU/HA ratio was about the same for purified "full" particles as for the crude lysates, the difference between 3T3- and 10A-grown virus was not a function of the number of "empty" particles. A comparative particles count in the electron microscope confirmed the

FIG. 2. Synthesis of viral DNA and viral capsid antigen in 10A and 3T3 cells infected by polyoma virus. Cells were infected at an MOI of 100 PFU/cell, and viral DNA synthesis was determined by the incorporation of ^3H -thymidine into polyoma viral DNA; frequency of cells synthesizing capsid antigen was determined by immunofluorescence. The left ordinate gives the cumulative counts/minute into viral DNA for 3T3 (●) or 10A (○) cells. The right ordinate gives the frequency of cells synthesizing V antigen determined at the times indicated for 3T3 (■) and 10A (□).

TABLE 2. Synthesis of viral DNA and infectious virus in 10A and 3T3 cells infected by polyoma virus^a

Expt	Cells	(A) Viral DNA		(B) Viral DNA		(C) Infectious virus		B/C
		Counts/min	Per cent	PFU	Per cent	PFU	Per cent	
I	10A	2,600	3.3	4×10^8	8	8×10^6	0.69	5×10^{-4}
	3T3	80,000	100	5×10^4	100	1.16×10^9	100	4.3×10^{-5}
II	10A	5,000	5	2×10^8	3.3	1.2×10^6	0.6	1.6×10^{-3}
	3T3	100,000	100	6×10^4	100	2×10^8	100	3×10^{-4}

^a Multiplicity of infection = 100 plaque-forming units (PFU)/cell. Cells were labeled with H^3 -thymidine from 15 to 31 hr after infection, and the DNA was extracted by the method of Hirt. Polyoma viral DNA was then further purified by band centrifugation. The infectivity of the viral DNA was determined as described. The yield of infectious virus was determined on parallel cultures at 48 hr after infection.

TABLE 3. *Infectivity of polyoma virus produced in 10A and 3T3 cells^a*

Cells	Crude virus lysate		Purified virus		
	PFU	HAU ^b	PFU	HAU	PFU/HAU
10A	7.5×10^8	30,000	3×10^8	40,000	7.5×10^3
10A	5.5×10^8	55,000	2×10^8	22,000	9×10^3
10A	7×10^7	26,000	3×10^7	16,000	2×10^3
10A	NT ^c	NT	4.5×10^8	120,000	3.8×10^3
3T3	9×10^{10}	400,000	3.5×10^{10}	450,000	8×10^4
3T3	NT	NT	3×10^{10}	300,000	10^5

^a Data are from six independent virus preparations. In all cases, cells had been infected at multiplicity of infection of 20 to 50 plaque-forming units (PFU)/cell of the same virus stock. Virus was harvested and purified as described.

^b HAU, hemagglutination units.

^c NT = not tested.

conclusion that polyoma 10A was about 90% non-infectious as compared to standard polyoma virus. The reduced infectivity appeared to be due to a defect in the nucleic acid component of the virus since extraction of the DNA from purified polyoma 10A did not lead to recovery of the infectivity (Table 4).

The properties of polyoma 10A particles were not grossly different from those of standard polyoma virus. Their nucleic acid was resistant to deoxyribonuclease, and their morphology appeared normal. In cesium chloride equilibrium density gradients, however, polyoma 10A particles banded at a density which was slightly lower than that of polyoma grown in 3T3 (Fig. 3). The difference was very small and, for some preparations, almost undetectable. Examination of the DNA extracted from purified polyoma 10A revealed the sedimentation pattern shown in Fig. 4. Only ~10% of the DNA molecules cosedimented with the supercoiled form of polyoma viral DNA (20S, form I; reference 21), whereas the majority of the molecules had a sedimentation coefficient of 16 to 14S. Extraction of the DNA from polyoma virus grown in 3T3 gave, as already described (19), 90 to 95% of form I and 5 to 10% of II and III.

The majority of the polyoma 10A DNA molecules banded as linear or nicked circular DNA in ethidium bromide, CsCl density gradients (16) (Fig. 4). In alkaline (pH 13) sedimentation gradients (21), they had a sedimentation coefficient of ~16S (Fig. 5). From these experiments, it was concluded that about 90% of the DNA molecules extracted from polyoma 10A were of linear structure, with an average molecular weight of $\sim 3 \times 10^6$ daltons (21), and electron microscopy confirmed their linear configuration. The remaining 10% of the molecules behaved as typical supercoiled polyoma DNA. It

is worth mentioning that changing the methods of extraction of DNA from polyoma 10A did not change the distribution of the polyoma 10A DNA molecules. In addition, when polyoma-infected 3T3 cells, which had been labeled with ^{14}C -TdR, were mixed with ^3H -labeled infected 10A cells, the virus harvested and purified, and the DNA extracted, 90% of the ^3H -polyoma 10A DNA molecules still sedimented at 16 to 14S, whereas 90% of the ^{14}C -polyoma 3T3 DNA molecules sedimented at 20S.

In CsCl equilibrium density gradients, the DNA extracted from polyoma 10A virus banded at a density slightly lower than that of polyoma DNA (from virus grown in 3T3 cells), more or less coincident with that of mouse cellular DNA (Fig. 6). This suggested that most of these molecules were of cellular origin (14, 24).

To determine precisely whether polyoma 10A DNA was viral or cellular in nature, DNA-DNA hybridization experiments were performed. On the basis of these experiments (Table 5), it was concluded that the total DNA extracted from polyoma 10A consisted of 10 to 15% viral DNA and 85 to 90% of cellular DNA. Polyoma 10A DNA was then fractionated by ethidium bromide, CsCl density gradients. As expected, the lower band (~10% of the radioactivity) consisted of viral DNA molecules. However, some DNA capable of hybridizing with viral DNA was still present among the linear molecules (upper band).

Polyoma 10A seems, therefore, to consist mainly of particles containing cellular DNA (pseudovirions; references 14, 24). It is interesting to note, however, that whereas 5 to 10% of the particles contain typical polyoma DNA, which is fully infectious, some viral DNA is also present among the linear, mainly cellular, DNA molecules. The state of these viral DNA sequences (whether they represent linear or nicked polyoma

TABLE 4. Infectivity of DNA extracted from polyoma virus grown in 10A or 3T3 cells

Cells	Infectivity of virus (PFU/ml) ^a	Infectivity of DNA (PFU/ml)	Infectivity DNA/virus
10A	7×10^6	6.5×10^2	9×10^{-5}
3T3	2.5×10^9	2×10^5	8×10^{-5}

^a PFU, plaque-forming units.

FIG. 3. Density of polyoma 10A particles. Purified "full" polyoma 10A, which had been labeled with ^3H -thymidine, was mixed with a ^{14}C -labeled preparation of polyoma virus grown in 3T3 cells. (In both cases the cells had been exposed to labeled thymidine starting after virus adsorption, and the virus was harvested 4 days later.) The virus was then subjected to equilibrium density gradient centrifugation in CsCl . The initial CsCl density was 1.30, 3 ml/tube; centrifugation was performed for 24 hr at 35,000 rev/min in the Spinco SW50.1 rotor (16°). Fractions were collected from the bottom of the tube, trichloroacetic acid-precipitated, and counted. Symbols: (●) ^{14}C polyoma 3T3; (○) ^3H polyoma 10A.

DNA molecules, which were not separated from the cellular DNA molecules, or whether they may be covalently linked with cellular DNA in some virus particles) is under investigation.

Biological activity of polyoma 10A. During the course of this investigation, the possibility arose that the noninfectious polyoma 10A could have been host-modified (12), so that it would have been fully infectious in 10A cells, even if not so in 3T3. This was not found to be the case. Polyoma 10A is largely noninfectious in any host cell tested. However, during the course of these experiments, evidence was obtained that the noninfectious polyoma 10A particles possess some biological activity. Table 6 shows the frequency of cells synthesizing T antigen or V antigen in a one-cycle growth experiment after infection with polyoma virus grown either in 3T3 or in 10A cells. Cells were infected with the same multi-

plicities (in PFU) of the two viruses. (This corresponds for polyoma 10A to a 10-fold excess of noninfectious virus particles.) While at low MOI the frequency of V-antigen producing cells was

FIG. 4. DNA extracted from polyoma 10A particles. 10A cells were infected with polyoma virus (50 PFU/cell) and exposed to ^3H -thymidine ($4 \mu\text{Ci/ml}$, 1.2 Ci/mmol) beginning after virus adsorption. The virus was harvested at 4 days and purified as described. 3T3 cells were treated in the same way except that they were labeled with ^{14}C -thymidine ($0.25 \mu\text{Ci/ml}$, 60 mCi/mmol). DNA was extracted from the purified virus preparations by SDS-NaCl. Samples of the two DNA preparations were mixed and subjected to band centrifugation (A), by layering 0.4 ml of the solution on 3.8 ml of CsCl , density 1.50, (pH 8.0) and centrifuging for 4 hr at 35,000 rev/min in the Spinco SW50.1 rotor. The DNA preparations were also subjected to dye buoyant density gradient centrifugation (B). CsCl and ethidium bromide were added to the DNA preparation and the final solution (2.6 ml, density 1.56, ethidium bromide $250 \mu\text{g/ml}$) was spun for 40 hr at 42,000 rev/min in the Spinco SW50.1 rotor. The ordinate gives the counts/min in the DNA of each fraction. Symbols: (●) ^{14}C -labeled polyoma 3T3 DNA; (○) ^3H -labeled polyoma 10A DNA.

FIG. 5. Alkaline sedimentation analysis of polyoma 10A DNA. (A) DNA extracted from purified polyoma 10A, which had been labeled with ³H-thymidine, was mixed with DNA extracted from polyoma virus grown in 3T3, which had been labeled with ¹⁴C-thymidine, and layered on 4 ml of CsCl (density 1.50, pH 13.0) which had been previously centrifuged for 1 hr at 35,000 rev/min. The tubes were then spun for 2.5 hr at 35,000 rev/min in the Spinco SW50.1 rotor. (B) polyoma 10A DNA, which had been labeled with ³H-thymidine, was mixed with a preparation of ¹⁴C-labeled polyoma viral DNA, which had been subjected to X-irradiation (25,000 r) to produce about 80% linear forms (19). The DNA mixture was then layered on 4 ml of CsCl (density 1.50, pH 13.0) and centrifuged for 4.5 hr at 35,000 rev/min in the Spinco SW50.1 rotor. Symbols: (○) ³H-labeled polyoma 10A DNA; (●) ¹⁴C-labeled polyoma 3T3 DNA in (A), ¹⁴C-labeled, X-irradiated polyoma DNA in (B).

about the same, at MOI of 5 PFU/cell polyoma 10A gave a frequency of V-antigen producing cells much higher than that given by standard polyoma virus. This complementation effect, although not strong, suggested that the non-infectious polyoma 10A particles contained a certain proportion of randomly defective viral DNA molecules, capable of complementing at high MOI. This was also suggested by the ratio of cells synthesizing T antigen to cells synthesizing V antigen obtained in the same experiment, which was about threefold higher for polyoma 10A than

FIG. 6. Density of polyoma 10A DNA. DNA extracted from purified polyoma 10A, labeled with ³H-thymidine, was mixed either with DNA from polyoma virus grown in 3T3 (A) or with 3T3 cellular DNA (B), both labeled with ¹⁴C. The DNA mixtures were then centrifuged to equilibrium in CsCl density gradients (average density 1.70). Centrifugation took place in the Spinco SW50.1 rotor for 48 hr at 40,000 rev/min at 20°. Fractions were collected from the bottom of the tube on Whatman fiberglass filters, dried, and counted. Symbols: (○) ³H-labeled polyoma 10A DNA; (●) ¹⁴C-labeled polyoma 3T3 DNA in (A), ¹⁴C-labeled 3T3 cell DNA in (B).

TABLE 5. Relative proportion of viral and host DNA sequences in the DNA extracted from polyoma (Py) 10A particles

³ H-Labeled DNA in solution	Counts/min incubated ^a	DNA immobilized on filters ^b	Counts/min bound to filters ^c	Per cent of input bound	Corrected values (%) ^d
Py DNA (form I) ^e	22,000 22,000	Py (form I) 10A cell	16,500 10	75 0.05	
10A Cell DNA	4,000 4,000	Py (form I) 10A cell	2 450	<0.1 11.2	
Py 10A DNA (total)	4,000 4,000	Py (form I) 10A cell	400 360	10.0 9.0	13.3 80
Py DNA (form I)	28,000 28,000	Py (form I) 10A cell	19,600 0	70 <0.05	
10A Cell DNA	16,500 16,500	Py (form I) 10A cell	30 2,800	0.18 16.9	
Py 10A DNA (linear or nicked) ^f	1,280 1,280	Py (form I) 10A cell	80 200	6.25 15.6	8.9 92

^a Specific activity of the DNA preparations used ranged from 100,000 to 200,000 counts per min per μ g. The amount of ³H-labeled DNA incubated was at least 10-fold smaller than the amount of DNA on the filters.

^b For Py DNA, 2 μ g; for 10A DNA, 5 μ g.

^c Number of counts/minute bound to blank filters was subtracted. Counts/minute in blanks never exceeded 0.15% of the input. Values of bound counts/minute are the average of duplicates.

^d Corrected values were calculated on the basis of the efficiency of the homologous reaction, i.e., viral DNA versus viral DNA, host DNA versus host DNA.

^e Supercoiled Py DNA purified on ethidium bromide, CsCl density gradients.

^f Purified on ethidium bromide, CsCl density gradients.

TABLE 6. Synthesis of T antigen and viral capsid antigen in 3T3 cells infected with polyoma virus grown in 10A or 3T3

Multi- plicity of infection (PFU/ cell)	PY3T3 ^a			PY10A ^b		
	Cells synthe- sizing T antigen ^c (%)	Cells synthe- sizing V antigen ^d (%)	T/V	Cells synthe- sizing T antigen ^c (%)	Cells synthe- sizing V antigen ^d (%)	T/V
50	90	52	1.7			
5	10	10	1	80	46.5	1.7
1	1.16	1.2	1	4.6	1.5	3
0.5	0.75	0.78	1	2.9	0.91	3.2
0.1	0.26	0.25	1	0.65	0.3	2.2

^a Purified virus grown in 3T3 cells.

^b Purified virus grown in 10A cells.

^c Determined at 24 hr after infection by immunofluorescence.

^d Determined at 32 hr after infection by immunofluorescence.

for polyoma grown in 3T3. Polyoma 10A contains, therefore, viral particles able to promote T-antigen synthesis but unable to complete the infectious cycle.

The transforming ability of BHK cells of

polyoma 10A (expressed as ratio transforming units/PFU) was, however, not significantly higher than that of standard polyoma virus. Reinfection of 10A cells, with polyoma 10A, (which is already 90% noninfectious) at high MOI, seemed to increase the proportion of noninfectious particles in the yield. This finding is undergoing further investigation.

Encapsidation of host DNA in 10A cells. Since the only step of the viral cycle which seems to be affected in 10A cells is DNA synthesis, it seems natural to think that the reduction of viral DNA synthesis and the extremely high encapsidation of cellular DNA into virions are related. Very little is known about what causes the reduction in polyoma DNA synthesis in 10A. The small amount of viral DNA synthesized is stable and, more important, apparently normal. Its efficiency of encapsidation is, however, very low. If the efficiency of encapsidation of the viral DNA is determined by first labeling the cells with ³H-thymidine at the time of maximum rate of viral DNA synthesis (24 to 32 hr), and then chasing with cold thymidine and determining the amount of labeled viral DNA found in mature virions, this value is in 3T3 on the order of 15%. In 10A

the efficiency of encapsidation is only 1 to 2%, since 90% of the total radioactivity found in the virions is represented by cellular DNA. Is there an effective competition between viral DNA and cellular DNA of the right molecular weight for encapsidation, which, owing to the low amounts of viral DNA present, leads to prevalent encapsidation of cell DNA? If competition between low-molecular-weight ($\sim 3 \times 10^6$) cellular DNA and polyoma DNA were the only cause of the phenomenon, one would expect to find a substantial accumulation of cellular DNA with a molecular weight of $\sim 3 \times 10^6$ daltons in the infected cells. This was never found to be the case. When 10A cells were labeled with ^3H -thymidine for 24 hr prior to infection to 35 or 40 hr after, to uniformly label all DNA, and the DNA was extracted, the amount of radioactivity in 16 to 14S pieces of cell DNA never exceeded that found in viral DNA and was generally smaller. Accordingly, the breakdown of cell DNA in 10A cells was not found to be higher than in 3T3 cells.

The cellular DNA found in polyoma 10A virions seemed to be preferentially that which had been at least partially synthesized after infection. This was shown by two types of experiments. (i) 10A cells were labeled prior to infection (~ 36 hr), and the label was removed at about 12 hr after infection (about the time when viral DNA synthesis begins). The amount of radioactivity found in the virus at the end of the infectious cycle was compared with that found in virus extracted from parallel cultures, which had been labeled pre- and postinfection, until the end of the infectious cycle. Little radioactivity (~ 10 to 15%) was found in the virions extracted from cultures labeled before the onset of viral DNA synthesis as compared to cultures continuously labeled. The converse type of experiment also gave equivalent results. Labeling the cells from 12 hr after infection gave approximately the same amount of radioactivity in the viral yield as that found in virus extracted from continuously labeled cells (pre- and postinfection). (ii) When 10A cells were labeled with ^3H -thymidine for 4 to 5 hr during the time of maximum viral DNA synthesis (procedure which should emphasize the labeling of viral DNA versus cellular DNA) and the label was then chased into mature virions, extraction of the DNA from these virions revealed the same pattern of radioactivity found in virus labeled under standard conditions: about 10% 20S, 90% 16 to 14S. These experiments, therefore, suggest that the polyoma 10A pseudovirions contain preferentially host DNA which has been at least partially synthesized after infection, more precisely during the same times in which viral DNA is synthesized.

DISCUSSION

Two main features characterize the multiplication of polyoma virus in the 10A hybrid line: the low amount of viral DNA synthesized upon infection and the unusually high proportion of particles containing cellular DNA in the yield. An inhibition of viral DNA synthesis has been observed in other mouse-hamster hybrid lines, and it is very likely due to an effect of the hamster genome (4). However, all of the other hybrids which exhibited a reduced level of viral DNA synthesis were chromosomally characterized by a relative excess of hamster chromosomes, accompanied by an approximately normal mouse complement (4). Hybrid 10A instead, which contains 32 biarmed and 72 telocentric chromosomes, belongs to the group of balanced hybrids, i.e., hybrids which contain approximately a parental complement of both hamster and mouse chromosomes. It has already been pointed out (4), however, that our classification of the hybrids could have been in some cases inadequate, due to the fact that individual chromosomes had not been identified. It is not unlikely, therefore, that 10A may be a "false" balanced hybrid, at least in respect to the chromosomes involved in permitting viral DNA replication, and that it may really belong to the same category of the other hybrids with reduced viral DNA synthesis. This hypothesis finds some support, not only in the characteristics of viral multiplication in 10A but also in the morphology of these cells, their generation time, etc., which all resemble more that of hybrids with an excess of hamster chromosomes than the balanced ones.

The possible mechanism by which an excess of hamster chromosomes in the hybrids could lead to an impairment of viral DNA synthesis was discussed in a previous paper (4). At any rate, since both in hybrid 10A and in other hybrids with similar behavior no other viral functions seems to be affected, it is likely that the peculiar composition of the viral yield in 10A is a direct consequence of the impairment of viral DNA synthesis. The virus produced in hybrid 8A, which also supports a reduced rate of viral DNA synthesis (4), similarly contains an excess of 14S DNA. Work is in progress to clarify the block of viral DNA synthesis which occurs in these cells. It is already known, however, that the small amount of DNA synthesized is stable and apparently normal. Its efficiency of encapsidation, however, which is on the order of 10 to 15% for 3T3 cells, is reduced to about 1% by the fact that 90% of the particles contain predominantly cellular DNA. This explains why 10A cells, which synthesize about 5% viral DNA of 3T3, eventually produce less than 1% infectious virus.

The simplest interpretation of this phenomenon

would be that the drastic reduction in the amount of viral DNA synthesized leads to an effective competition for encapsidation between polyoma DNA and low-molecular-weight cellular DNA, which, being in excess, succeeds in constituting the nucleic acid of most of the particles. This mechanism would imply that any DNA molecule of approximately the right molecular weight has the same probability of being enclosed in polyoma capsids, regardless of its structure or base sequence. However, since we failed to demonstrate any accumulation in infected 10A cells of small-size host DNA, in excess of polyoma DNA, other alternative hypotheses could be considered. For example, it is conceivable that DNA of any molecular weight could compete for encapsidation, if a mechanism existed for cutting it to the proper size during the packaging process (10). Short of experimental evidence, this is at present a mere hypothesis. However, if polyoma DNA became integrated into the host DNA during its multiplication cycle, excision could be coupled with encapsidation, and a defect of the excision mechanism in 10A could explain the peculiar characteristics of the viral yield in this hybrid, namely, the encapsidation of abnormal proportions of cellular DNA and the fact that some viral DNA sequences are still present among the linear host DNA molecules extracted from polyoma 10A pseudovirions.

It can be also asked whether the properties of the polyoma 10A noninfectious particles may be different from those of the pseudovirions (14, 24) which are found in mouse cells. In fact, (i) the DNA extracted from polyoma 10A particles had a rather homogenous distribution around a molecular weight of 3×10^6 daltons, in contrast to the pseudovirions described by Michel et al. (14), which seemed to contain DNA variable in size (ii) Polyoma 10A contains preferentially host DNA which has been at least partially synthesized after infection. This fact could be related to the observation (5, 6) that host DNA synthesized after infection is preferentially broken down in mouse cells. (iii) Polyoma 10A pseudovirions possessed some biological activity; part of them seemed to be able to induce T antigen, and they gave rise to a weak complementation. These last data are in agreement with the DNA-DNA hybridization data, which showed that the purified 14S fraction of polyoma 10A DNA still contained some viral DNA sequences.

It is possible that these differences are not of real significance. With regard to (i), in fact, the specialized situation in 10A makes it easier to analyze the bulk of the pseudovirions, whereas in mouse cells only the "light" pseudovirions can be purified satisfactorily (14, 24). Statement (ii) does not contradict what has been found by other

authors, since they did not perform the same type of experiments; with regard to (iii), both Winocour (24) and Michel et al. (14) showed only that pseudovirions DNA was mainly cellular and did not investigate T-antigen synthesis or complementation effects.

It is likely, therefore, that the 10A pseudovirions are not substantially different from those found in mouse cells. The fact that 10A cells produce a yield which is at least 90% composed by pseudovirions facilitates the investigation of the nature and the formation of these particles. Work is in progress to clarify further their properties and the mechanism of host DNA encapsidation.

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