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Acquisition of Sequences Homologous to Host Deoxyribonucleic Acid by Closed Circular Simian Virus 40 Deoxyribonucleic Acid

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The synthesis of closed circular simian virus 40 (SV40) deoxyribonucleic acid (DNA) containing sequences homologous to host cell DNA depends upon the conditions under which the cells are infected. When BS-C-1 monkey cells were infected with non-plaque-purified virus at low multiplicity of infection [MOI, 0.032 plaque-forming units (PFU)/cell], little, if any, of the SV40 DNA extracted from the infected cells hybridized to host DNA; but when increasingly higher multiplicities were used (in the range 0.16 to 3,000 PFU/cell), an increasingly greater amount of the extracted SV40 DNA hybridized to host DNA. The same effect was observed when the closed circular SV40 DNA was extracted from purified virions (grown at low and high MOI) rather than from the infected cell complex. When the cells were infected at high MOI with plaque-purified virus (11 viral clones were tested), none of the SV40 DNA extracted from the cells hybridized detectably with host cell DNA. However, plaque-purified virus that was serially passaged, undiluted, induced the synthesis of virus DNA which again showed extensive homology to host DNA. It is suggested that, under certain circumstances, recombination occurs between viral and host DNA during lytic infection which results in the incorporation of host DNA sequences into closed circular SV40 DNA.

The process of cell transformation by deoxyribonucleic acid (DNA)-containing oncogenic viruses is now known to be accompanied by the integration of viral DNA into cellular chromosomal DNA (reviewed in reference 26). It is not known, however, if recombinational events between viral and cellular DNA can occur during the lytic infection of cells by these viruses. It was shown previously that purified simian virus 40 (SV40) virions, grown in the BS-C-1 line of monkey cells, contain closed circular viral DNA molecules which hybridize to host cell DNA, and it was suggested that host DNA sequences may have been covalently incorporated into SV40 DNA molecules during the lytic infection (1). In the present study we will show that the type of SV40 DNA synthesized in BS-C-1 cells depends upon the conditions under which the cells are infected. Virus DNA which hybridizes detectably to host DNA is not produced in cells infected with plaque-purified virus, or in cells infected with non-plaque-purified virus at low multiplicity of infection (MOI). On the other hand, viral DNA molecules containing sequences homologous to cell DNA are produced in cells infected with serially passaged plaque-purified virus, or with non-plaque-purified virus at high MOI. The results supply strong support for the notion that SV40 DNA molecules can, under certain conditions, acquire host DNA sequences during the lytic cycle of multiplication. It is suggested that this acquisition results from a recombination event between cellular and viral DNA during lytic infection, and a model which accounts for the mode of production of SV40 DNA molecules containing cell DNA sequences will be discussed.

MATERIALS AND METHODS

Cells. The BS-C-1 line of African green monkey kidney cells (11) was obtained from Flow Laboratories. The cells were cultured in plastic dishes in Eagle's medium with a fourfold concentration of amino acids and vitamins, supplemented with 10% calf serum. At the end of 10 to 15 tissue culture passages, the cells were discarded and new cultures were started with a fresh sample of cells from an early passage of the original population (stored at −90 C). Periodic tests for the presence of contaminating SV40, performed by using an immunofluorescence test (7)

for the SV40-induced nuclear tumor antigen, were consistently negative.

Infection of cells. The cell layer (containing 4×10^6 cells) was infected by adding 1 ml of virus diluted in medium with 2% calf serum. The MOI refers to the amount of virus added (the amount of virus adsorbed was not determined) and is expressed as the number of plaque-forming units (PFU) per cell. A 2-hr period was allowed for virus adsorption. The cell layer was then covered with 8 ml of medium containing 2% calf serum. The infected cells were labeled by adding ^{3}H-Tdr (thymidine-*methyl*-^3H, 9 to 23 Ci/mmole, obtained from the Radiochemical Centre, Amersham, England) at a concentration of 10 μCi/ml of medium. The labeling periods are noted below.

Virus. Strain 777 (6) of SV40 was used throughout. Virus titers were determined by plaque assay on BS-C-1 cells (20). Non-plaque-purified stocks of virus were prepared by infecting cells at a MOI of 0.2 PFU/cell. To obtain plaque-purified virus stocks, single plaques were picked from assay plates containing one or two plaques only. These isolates were plaque-purified twice more. High titer stocks of the third passage plaque isolates were made by infecting BS-C-1 cells at very low MOI (5×10^{-4} PFU/cell).

To purify the virus, the infected cell lysates were subjected to sonic vibration (3 min at full power in a 10 kc/sec Raytheon sonic vibrator) and treated with sodium deoxycholate (0.1% final concentration; 30 min at 37 C). After low-speed centrifugation, 20-ml samples of the supernatant fluid were layered on top of 9-ml samples of CsCl solution (density of 1.40 g/ml) and centrifuged in a Spinco SW25 rotor at 22,000 rev/min for 3.5 hr. The lowest band of particles in the CsCl cushion was collected and, after adjustment of the CsCl density to 1.34 g/ml, centrifuged to equilibrium at 35,000 rev/min for 20 hr in a Spinco SW50 rotor. The lowest band of virus was collected and dialyzed against Eagle's medium. This purified virus was used for the infection of cells as specified below. Larger amounts of virus for DNA extraction were purified by a similar procedure except that the crude virus lysates were treated with 0.25% trypsin and 0.5% sodium deoxycholate (2). After concentration by sedimentation (100,000 $\times$ *g*, 3 hr) the virus was centrifuged to equilibrium in CsCl density gradients as above.

Extraction and purification of closed circular SV40 DNA. Viral DNA was extracted from ^{3}H-TdR-labeled, infected cells by the selective procedure of Hirt (10). Closed circular viral DNA was purified by band sedimentation in alkaline CsCl solution (21). The nucleic acids in the soluble fraction of the extract prepared by Hirt's procedure were precipitated with ethanol and resuspended in alkaline phosphate buffer (0.1 M dibasic sodium phosphate adjusted with KOH to *p*H 12.8). After 1 to 2 hr at room temperature, 0.2-ml samples of the material were layered on top of 3 ml of alkaline CsCl solution (density 1.52 g/ml, made up in alkaline phosphate buffer, *p*H 12.8) and centrifuged at 35,000 rev/min for 100 min (at 20 C) in a Spinco SW50 rotor. The closed circular viral DNA sedimented as a sharp band in the 53*S* region of the gradient (22). This fraction was collected and dialyzed exhaustively against 0.01$\times$ SSC [1$\times$ SSC, or standard saline citrate, is 0.15 M NaCl, 0.015 M sodium citrate (13)]. Control experiments with similarly treated extracts from uninfected cells, or from infected cells labeled from 3 to 12 hr postinfection, showed that the 53*S* region of the alkaline-CsCl gradient was not contaminated with cellular DNA. Further fractionation of the closed circular viral DNA by equilibrium centrifugation in CsCl gradients supplemented with ethidium bromide (17) produced no change in its ability to hybridize with cellular DNA. DNA was extracted from purified virus with phenol-sodium trichloroacetate (23) and was purified by band sedimentation in alkaline CsCl solution as above.

DNA-DNA hybridization in formamide. BS-C-1 cell DNA and *Escherichia coli* DNA were prepared as described elsewhere (1). The cell and bacterial DNA species were denatured by alkali treatment (0.3 N KOH for 1 hr at room temperature); closed circular SV40 DNA was denatured by boiling for 15 min in 0.01$\times$ SSC followed by rapid cooling in ice water. Samples (50 μg) of the denatured cell or bacterial DNA and 10-μg samples of the denatured SV40 DNA were immobilized on nitrocellulose membrane filters (Millipore HA 0.45 μm) according to the procedure of Gillespie and Spiegelman (8). Before use in hybridization reactions, the DNA-containing filters, or blank control filters, were preincubated at 60 C for 3 to 6 hr in a solution containing 3$\times$ SSC and 0.04% bovine serum albumin (4).

Closed circular SV40 ^{3}H-DNA was boiled for 15 min in 0.01$\times$ SSC and rapidly cooled in ice water. By this treatment, the DNA was both fragmented and denatured. The fragmented DNA strands sedimented with a value of approximately 10*S* in alkaline 5 to 20% sucrose gradients (*p*H 12.8). This sedimentation value corresponds to a molecular weight of 500,000 daltons (19), which is about 30% the molecular weight of an intact SV40 DNA strand (ca. 1.5×10^6 daltons). The average fragment size was uniform from experiment to experiment.

Hybridization of the SV40 ^{3}H-DNA fragments was carried out in 50% formamide (Puriss grade from Fluka, AG) at 37 C (14). The denatured ^{3}H-DNA was diluted 1:1 with 100% formamide, and 0.1-ml samples of the mixture were added to scintillation vials which contained one preincubated filter and 0.9 ml of hybridization buffer [50% (v/v) formamide; 0.75 M NaCl; 0.5% (w/v) sodium dodecyl sulfate; 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride; final *p*H adjusted to 7.4]. The vials were incubated at 37 C in a water bath shaker for 18 to 22 hr, unless otherwise stated. At the end of the incubation period, the radioactive solution was removed from the vials by suction, and the filters were washed with three 3-ml portions of hybridization buffer. The filters were then transferred to new vials containing 3 ml of hybridization buffer and incubated for 1 hr at 37 C. Finally, the filters were washed with four 10-ml portions of 1$\times$ SSC, dried, and counted in a toluene-based scintillation liquid. The amount of DNA immobilized on the filter was at least 50-fold greater than the amount of ^{3}H-DNA in solution. All hybridization reactions were carried out in duplicate.

To recover the hybridized ^{3}H-DNA, the filters were incubated at 37 C for 1 hr in 3 ml of elution buffer (90 volumes of 100% formamide, 9 volumes of distilled water, and 1 volume of a solution containing 0.001 M ethylenediaminetetraacetic acid, 0.1 M NaCl, 0.01 M Tris-hydrochloride, and 1%, w/v, sodium dodecyl sulfate). The final *p*H of the elution buffer was adjusted to 9.0 with 3 N HCl. Over 90% of the hybridized ^{3}H-DNA was recovered from the filters.

RESULTS

Effect of MOI on the synthesis of viral DNA containing sequences homologous to host cell DNA. The objective of the first experiment was to determine the homology to cell DNA of the closed circular SV40 DNA molecules extracted from cells infected with non-plaque-purified virus at different MOI. Groups of BS-C-1 cultures were infected with the virus at multiplicities in the range 3,000 to 0.032 PFU/cell (Table 1). The infected cells were labeled with ^{3}H-Tdr starting at 24 or 30 hr postinfection. At the end of the labeling period (up to 5 days was required to obtain a sufficient amount of radioactive SV40 DNA in the case of cultures infected at very low MOI), the radioactive virus DNA was selectively extracted from the infected cells and purified by band sedimentation in alkaline CsCl solution to ensure that only closed circular DNA was present. The different ^{3}H-SV40 DNA preparations were then tested for their relative ability to hybridize with host cell DNA.

It will be noted from the data in Table 1 that the production of closed circular SV40 DNA homologous to host cell DNA was strikingly dependent upon the MOI used to initiate the infection. At low MOI (0.032 and 0.16 PFU/cell), the amount of ^{3}H-SV40 DNA bound to cell DNA filters was only two- to threefold greater than the background level to control filters. However, when a higher range of multiplicities was used (4 to 3,000 PFU/cell), the relative proportion of SV40 DNA which hybridized to cell DNA increased as the MOI was increased. In cells infected at a multiplicity of 3,000 PFU/cell, at least 28% of the newly synthesized viral DNA molecules contained regions which hybridized to cell DNA. The results presented in Table 2 show that the same MOI effect was observed when the closed circular DNA was extracted from purified virions rather than from the infected cell complex.

Virus DNA produced in BS-C-1 cells infected at high MOI with plaque-purified clones of SV40. The previous experiment, which indicated that the occurrence of closed circular SV40 DNA containing sequences homologous to cell DNA was dependent upon the MOI used to initiate the infection, was carried out with uncloned (non-plaque-purified) virus. The following experiment was performed to determine whether plaque-purified virus would induce, at high MOI, the synthesis of SV40 DNA molecules which hybridize to cell DNA. Eleven plaque-purified clones of SV40 were prepared by three successive plaque purification steps and grown to higher titer stocks starting from a very dilute inoculum (5×10^{-4} PFU/cell). These cloned virus preparations were purified and then used to infect a series of BS-C-1 cultures at a uniformly high MOI of

TABLE 1. *Homology to host DNA of closed circular SV40 DNA extracted from BS-C-1 cells infected at different multiplicities of infection (MOI) with non-plaque-purified virus*

MOI (PFU/cell)[a]	Labeling time (hr postinfection)	^{3}H input to hybridization reaction (counts/min)	Per cent ^{3}H-DNA bound to filters containing:			Per cent bound:[b] BS-C-1/SV40
			SV40 DNA	BS-C-1 DNA	No DNA	
3,000	24–48	16,450[c]	69.1	28.0[d]	0.15	42.0
100	30–54	17,230	67.3	14.1	0.40	21.0
20	30–54	17,860	70.1	7.3	0.30	10.3
4	30–63	18,600	51.2	2.4	0.30	4.7
0.16	30–149	16,681	52.2	0.7	0.20	1.3
0.032	30–149	12,048	73.0	0.5	0.20	0.7

[a] Plaque-forming units.

[b] (Per cent bound to BS-C-1 DNA/percent bound to SV40 DNA) × 100. This index expresses the ability of SV40 ^{3}H-DNA to hybridize with cell DNA, corrected for the efficiency of hybridization shown by the control SV40 ^{3}H-DNA–SV40 DNA reaction.

[c] The size of the SV40 ^{3}H-DNA fragments used in this and all subsequent experiments was approximately one-third the size of an intact SV40 DNA strand (see text).

[d] Since no nuclease digestion was used to remove nonpaired regions in the hybrid complex, the figures in this column reflect the proportion of molecular fragments which contain sequences homologous to cell DNA and not the proportion of such sequences in any one molecular fragment.

TABLE 2. *Homology to host DNA of closed circular DNA extracted from non-plaque-purified SV40 virions grown at high and low multiplicities of infection (MOI)*[a]

MOI (PFU/cell)[b]	^{3}H input to hybridization reaction (counts/min)	Per cent ^{3}H-DNA bound to filters containing:				Per cent bound: BS-C-1/SV40
		SV40 DNA	BS-C-1 DNA	*E. coli* DNA	No DNA	
100	30,000	89.7	13.4	0.30	0.10	15.0
0.05	32,265	82.0	2.4	0.15	0.10	2.9
0.00001	15,500	75.4	1.0	NT[c]	0.20	1.3

[a] BS-C-1 cell cultures were infected with a non-plaque-purified stock of SV40 at the MOI noted above. The infected cells were labeled with ^{3}H-Tdr (on day 1 postinfection at high MOI; on day 4 postinfection in the case of the lower MOI) and maintained until cell lysis had occurred. The ^{3}H-labeled virus was harvested and purified, and the closed circular ^{3}H-DNA was extracted from the virions and purified by sedimentation through alkaline CsCl solutions. The specific activities of the three viral DNA preparations were in the range of 100,000 to 180,000 counts per min per μg of DNA. The viral DNA preparations were tested for their relative ability to hybridize with filters containing 10 μg of SV40 DNA, 50 μg of BS-C-1 cell DNA, 50 μg of *Escherichia coli* DNA, or no DNA, as described in the text.

[b] Plaque-forming units.

[c] NT, not tested.

100 PFU/cell. As a control, a group of parallel BS-C-1 cultures were infected at the same MOI with purified virions from a non-plaque-purified stock of SV40. All cultures were labeled with ^{3}H-Tdr from 24 to 48 hr postinfection. The ^{3}H-SV40 DNA species were selectively extracted from the infected cells, purified by sedimentation through alkaline CsCl solutions, and tested for their relative capacity to hybridize with host cell DNA.

The results are presented in Table 3. It will be noted that none of the SV40 DNA preparations, which arose from the use of cloned virus, hybridized significantly with cell DNA (relative to the background level of hybridization with control filters). Similarly, no closed circular SV40 DNA with detectable homology to cell DNA appeared in cells multiply infected with a mixture of all 11 virus clones; nor was homology to cell DNA detected when the input to a single hybridization reaction was adjusted to contain equal amounts of each of the 11 SV40 DNA preparations induced by cloned virus. In sharp contrast, uncloned virus, at the same MOI, initiated the production of closed circular SV40 DNA which hybridized extensively to cellular DNA.

Virus DNA produced in BS-C-1 cells infected with undiluted, serially passaged, plaque-purified virus. In view of the results described in the previous section, it was of interest to determine the conditions under which plaque-purified virus would regain the ability to induce the production of closed circular viral DNA which hybridizes to host DNA. Undiluted, serial passage of the plaque-purified virus appeared to be a likely condition. To examine this, virus was isolated from a single plaque (third plaque purification

TABLE 3. *Lack of homology to host DNA of closed circular SV40 DNA extracted from BS-C-1 cells infected at high multiplicity of infection (MOI) with plaque-purified clones of virus*

Virus used for infection[a]	^{3}H input to hybridization reaction (counts/min)	Per cent of ^{3}H-DNA bound to filters containing:		
		SV40 DNA	BS-C-1 DNA	No DNA
Uncloned	17,995	53.6	14.0	0.60
Clone D	19,494	85.1[b]	0.56	0.68
Clone F	20,648	92.0	0.47	0.54
Clone H	23,921	98.5	0.61	0.59
Clone K	19,511	100.0	0.52	0.66
Clone II	18,570	100.0	0.38	0.65
Clone B	19,669	47.0	0.20	0.20
Clone C	21,377	42.2	0.30	0.48
Clone E	18,988	46.1	0.40	0.27
Clone G	23,667	38.6	0.31	0.32
Clone I	19,588	43.8	0.39	0.24
Clone J	18,617	46.5	0.43	0.36
Mixture of 11 clones[c]	49,515	35.0	0.37	0.26
	68,291[d]	33.0	0.33	0.37

[a] The MOI was 100 plaque-forming units (PFU)/cell in each case.

[b] In the hybridization tests on the viral DNA made in cells infected with clones D, F, H, K, and II, the hybridization incubation period was 44 hr; in all other cases, the incubation period was 18 to 22 hr.

[c] The cells were infected with a mixture containing equal amounts of each of the 11 clones, adjusted to a total MOI of 100 PFU/cell.

[d] The input to this hybridization reaction consisted of a mixture of equal amounts of the 11 SV40 DNA preparations (induced by each of the 11 clones).

step) and grown to a high-titer stock starting from a very low MOI (5×10^{-4} PFU/cell). As shown for the data on clone B in Table 3, this virus preparation did not induce, even at high MOI, the production of SV40 DNA with detectable homology to host DNA. The cloned stock of virus was then used to initiate a series of successive undiluted passages. Between each successive passage, the total yield of virus from the cultures was assayed for the number of plaque-forming particles and used undiluted (i) to infect cells for the next serial passage and (ii) to infect cells from which closed circular SV40 ^{3}H-DNA was subsequently extracted for hybridization tests. The results are shown in Table 4. It will be noted that as a result of successive undiluted passage, the plaque-purified virus acquired the ability to induce the production of SV40 DNA which hybridized to host cell DNA. The production of these viral DNA molecules progressively increased with each successive passage of the inoculum. It is also of interest to note (column 2, Table 4) that successive undiluted passage of the inoculum was accompanied by a sharp drop in the yield of plaque-forming particles.

Additional evidence for linkage between virus-specific and host cell-specific DNA sequences. The previous conclusion that SV40 DNA molecules contain covalently linked host DNA sequences was based upon the finding that sedimentation of the viral DNA in alkaline CsCl solutions and equilibrium centrifugation of the viral DNA in CsCl gradients supplemented with ethidium bromide failed to remove the component which hybridized with host DNA (1). The present findings suggested an additional test of this conclusion. If linkage between virus-specific and cell-specific DNA sequences exists in some SV40 DNA molecules, then those molecular fragments which hybridize to host DNA should rehybridize with high efficiency back to plaque-purified SV40 DNA which shows no detectable homology to cell DNA. To carry out this test, two types of SV40 DNA were prepared. ^{3}H-DNA was extracted from labeled virions harvested from cells infected with non-plaque-purified virus at high MOI; non-radioactive viral DNA, for immobilization on filters, was extracted from a population of plaque-purified virions that had never been serially passaged at high MOI (clone B, Table 3). The hybrids formed between the radioactive viral DNA fragments (approximately one-third the size of an intact SV40 DNA strand) and host cell DNA were disrupted with 90% formamide, and the ^{3}H-DNA fragments so recovered were tested for their ability to rehybridize back to filters containing the plaque-purified SV40 DNA. The results are shown in Table 5, where it can be seen that the ^{3}H-DNA fragments recovered from the hybrid complex with host DNA rehybridized (reaction 2b) back to plaque-purified SV40 DNA with a high efficiency that was fully comparable to that of the ^{3}H-DNA fragments recovered from the control SV40 DNA-SV40 DNA hybrid complex (reaction 2a). These results confirm the conclusion that the virus-specific and host-specific DNA sequences are covalently linked.

DISCUSSION

We have shown that the synthesis of closed circular SV40 DNA which hybridizes extensively to host cell DNA was dependent upon (i) the MOI, (ii) whether or not the infecting virus was plaque-purified, and (iii) the number of undiluted

TABLE 4. *Homology to host DNA of closed circular SV40 DNA extracted from BS-C-1 cells infected with undiluted, serially passaged, plaque-purified virus*[a]

Virus used for infection		^{3}H input to hybridization reaction (counts/min)	Per cent ^{3}H-DNA bound to filters containing:			Per cent bound: BS-C-1/SV40
Passage no.	Yield of plaque-forming particles (PFU/ml)		SV40 DNA	BS-C-1 DNA	No DNA	
1	10^9	15,452	65.6	0.7	0.4	1.1
2	10^9	21,346	63.0	1.3	0.4	2.1
3	10^8	20,357	50.8	3.5	0.4	6.9
4	3×10^6	15,156	55.0	10.6	0.7	19.3

[a] Passage 1 was initiated by infecting cells with clone B virus (Table 3) at 100 plaque-forming units (PFU)/cell. The infected culture was maintained until total lysis of the cells had occurred. The virus harvested from this culture was then divided into three samples: one sample was used undiluted to infect cells for the next passage; the second sample was used undiluted to infect another culture from which closed circular SV40 DNA was subsequently extracted for homology tests (the infected cells were labeled with ^{3}H-Tdr from 24 to 48 hr postinfection); the third sample was used for the determination, by plaque assay, of the number of plaque-forming particles. At each successive passage, the same procedure was repeated.

TABLE 5. *Rehybridization to plaque-purified SV40 DNA of SV40 DNA fragments which were hybridized to BS-C-1 cell DNA*

^{3}H-DNA in solution	^{3}H input per reaction (counts/min)	Per cent ^{3}H counts/min bound to filters containing:		
		SV40 DNA[a]	BS-C-1 DNA	No DNA
1. SV40[b]	30,400 (=0.047 μg)	90.1	14.7	0.1
2.[c] (a) Eluted from SV40 DNA filters	12,750	51.2	13.8	4.7
(b) Eluted from BS-C-1 DNA filters	3,150	66.5	20.0	0.7

[a] Derived from plaque-purified virus (clone B, Table 3).

[b] Derived from virus grown in cells infected with non-plaque-purified virus at 100 plaque-forming units/cell (the infected cells were labeled with ^{3}H-Tdr during days 1 through 5 postinfection).

[c] The SV40 ^{3}H-DNA fragments bound, in reaction 1, to plaque-purified SV40 DNA (three filters) or BS-C-1 DNA (four filters) were recovered as described in the Materials and Methods section, precipitated out of the formamide elution buffer with ethanol, resuspended in 0.01 × SSC, and tested for their capacity to rehybridize back to plaque-purified SV40 DNA or BS-C-1 cell DNA.

serial passages of the plaque-purified virus. When non-plaque-purified virus was used as inoculum, the production of viral DNA containing sequences homologous to cellular DNA increased as the MOI was increased. Viral DNA homologous to cell DNA was not detected in cells infected at high MOI with 11 plaque-purified viral clones. However, plaque-purified virus after serial, undiluted passage induced the production of viral DNA which again hybridized extensively to host DNA. In agreement with previous results (1), evidence for linkage between viral DNA sequences and host homologous DNA sequences is supplied by the rehybridization experiment shown in Table 5; SV40 DNA of the type which hybridized to cell DNA rehybridized with high efficiency back to plaque-purified SV40 DNA of the type which shows no homology to cell DNA.

The radioactive SV40 DNA fragments used in the hybridization tests were of uniform size (approximately one-third the size of an intact SV40 DNA strand) in each experiment. No nuclease digestion was used to remove nonpaired regions in the hybrid complex. Hence, the figures shown in Tables 1 through 5, for the per cent hybridization between the SV40 DNA fragments and the cell DNA, are *relative* values by which the proportion of molecules containing sequences homologous to cell DNA may be compared in different viral populations. An estimate of the proportion of sequences homologous to cell DNA in any one molecule cannot be deduced from the experiments reported herein.

Results which differ from those noted above have been reported by Gelb et al. (5). These authors failed to find any evidence for extensive homology between BS-C-1 host cell DNA and closed circular DNA from plaque-purified but serially passaged SV40 virions. Possibly a low MOI during serial passage of the virus, or differences in the strains of virus and host BS-C-1 cells used, may account for the different results obtained. On the basis of DNA reassociation kinetics, however, Gelb et al. (5) found evidence for the presence of a small amount of SV40 complementary sequences in normal African green monkey DNA (an average amount equivalent to 0.5 SV40 genome per cellular genome). It remains to be seen whether or not this finding is related to the observations reported here.

From electron microscopy studies of complexes formed by self-annealing of SV40 DNA strands (obtained by the introduction of a single break into double-stranded, closed circular DNA), Tai, Smith, Sharp, and Vinograd (J. Virol. 9: 317–325) have shown that some of the closed circular DNA molecules extracted from SV40 virions passaged at high MOI contain a region of substituted DNA in addition to deletions. The substituted DNA was found to be, on the average, about 20% of the length of SV40 DNA. Such molecules were not detected in virion populations grown at low MOI. The conditions of infection which result in the formation of substituted SV40 DNA molecules are strikingly similar to those which result in the formation of SV40 DNA molecules containing sequences homlogous to cell DNA; and as suggested by Tai, Smith, Sharp, and Vinograd, it is highly likely that the substituted SV40 DNA molecules described by them contain host cell DNA.

With serial undiluted passage of the plaque-purified virus, the production of viral DNA containing sequences homologous to cell DNA increased at each successive passage. It is interesting to note that this increase in the production of

viral DNA homologous to cell DNA was accompanied by a sharp decrease in the yield of plaque-forming particles (see Table 4). Yoshiiki (27) has observed that undiluted passage of SV40 results in the production of non-plaque-forming particles containing closed circular DNA which is heterogeneous in size and slightly shorter than the DNA of the plaque-forming particles. Preliminary experiments in this laboratory indicate that the closed circular viral DNA molecules which hybridize to cell DNA are slightly shorter than the viral DNA molecules which show no homology to cell DNA. The plaque-forming ability of these shorter molecules is currently being studied. If the SV40 DNA molecules which hybridize to cell DNA are defective, then the lack of detectable homology to cell DNA of the plaque-purified virus DNA can be attributed simply to the fact that defective molecules are eliminated during the plaque purification procedure. At the present time, however, we cannot exclude the possibility that some SV40 DNA molecules, capable of independent replication, contain a region which is homologous to a unique (3) segment of the cellular genome. The detection of this unique region in plaque-purified SV40 DNA—where each molecule would contain an identical stretch of sequences complementary to unique host DNA—would be virtually impossible under the hybridization conditions used (15).

We consider now a possible explanation which accounts for the formation of viral DNA molecules containing sequences homologous to host DNA, and for the observations that the production of these molecules increases progressively with each successive passage of plaque-purified virus, or with increasing MOI in the case of non-plaque-purified virus. We assume, first, that SV40 DNA molecules which contain sequences homologous to cell DNA are formed as a result of a recombination event between cellular and viral DNA. If the recombination event involves intact chromosomal DNA, covalently linked cell and viral DNA could arise by excision of the "integrated" viral DNA. We next assume that plaque-purified virus DNA has a low probability of recombining with cell DNA (perhaps because genetically homogeneous viral DNA can interact with only a few sites on the cellular genome), but that virus DNA which has acquired host DNA sequences during a previous infection has an enhanced probability of recombining with cell DNA in a subsequent infection. Serial undiluted passage of plaque-purified virus would thus increase the frequency with which the recombination event occurs and result in the emergence of progressively larger numbers of viral DNA molecules containing sequences homologous to cell DNA. Viral DNA molecules which have acquired sequences homologous to cell DNA may replicate by complementation with "normal" virus multiplying in the same cell. Hence the increased production of viral DNA containing sequences homologous to cell DNA in cells infected with serially passaged, plaque-purified virus (and in cells infected with non-plaque-purified virus at high MOI) may well be due to two separate effects; namely, an increase in the frequency with which the recombination event occurs, and an increase in the replication of the hybrid viral-cell DNA molecules which result from the recombination event.

It should be noted that the proposed recombination event between viral and cellular DNA during lytic infection is not necessarily related to the chromosomal integration of viral DNA which accompanies the transformation process (18). Nevertheless, it will be of interest to determine whether SV40 DNA which has acquired host DNA sequences has enhanced transforming activity. The possible use of polyoma and SV40 pseudovirions for the transduction of mammalian genes has been suggested (9, 12, 16, 24, 25). The linear host DNA in polyoma and SV40 pseudovirions, however, is generally unlinked to viral genome DNA. Closed circular SV40 DNA which contains host DNA appears to be a more promising candidate for transducing mammalian genes since the linked viral DNA may supply essential integration functions.

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