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Integration of Simian Virus 40 Deoxyribonucleic Acid into the Deoxyribonucleic Acid of Permissive Monkey Kidney Cells

K. HIRAI AND V. DEFENDI
The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

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Integration of simian virus 40 (SV40) deoxyribonucleic acid (DNA) into cellular DNA occurred when permissive African green monkey kidney (CV-1) cells were infected at a low multiplicity of SV40 in the presence of cytosine arabinoside.

In several cell systems it has been shown that simian virus 40 (SV40) deoxyribonucleic acid (DNA) is integrated into the cellular DNA of SV40-transformed cells (10). We have recently demonstrated that SV40 DNA becomes associated in an alkali-stable form to the DNA of Chinese hamster embryo cells at 15 to 20 hr postinfection (p.i.) when cellular DNA synthesis and T antigens are induced (5).

In the cell systems which have been analyzed for integration, SV40 infection is essentially nonpermissive since virus replication occurs, if at all, in only a small fraction of the cell population. In the present study we have examined whether SV40 DNA is integrated into the DNA of permissive African green monkey kidney (CV-1) cells. In this system the detection of integration of viral DNA into the cellular DNA is complicated by the fact that the viral DNA does replicate. Thus the possibility of nonspecific trapping of SV40 DNA in the viscous fast-sedimenting cellular DNA represents a serious obstacle to the interpretation of the results. This possibility, however, can be minimized by taking advantage of the fact that the SV40 DNA integration event is not effected by inhibition of DNA synthesis (5), as demonstrated in the case of the Chinese hamster cells. Thus, the interference of SV40 DNA progeny in the permissive cells can be excluded.

In these experiments, confluent monolayers of CV-1 cells were infected with SV40 at a multiplicity of infection of 0.5 plaque-forming units (PFU) per cell. The cultures were split at the ratio of 1:2 and cultured in the presence of D-arabinosyl cytosine (Ara-C) at a concentration of 15 μg/ml. At various times thereafter, nuclei were isolated by the Penman method (9) and DNA was extracted by the selective method of Hirt (6). The DNA from the supernatant fraction, containing SV40 and fragmented DNA, and from the sedimented fraction, containing nuclear DNA, was purified by the *p*H phenol method and CsCl equilibrium density centrifugation (5) and subjected to DNA-ribonucleic acid (RNA) hybridization as previously described (4).

Association of the SV40 DNA to the CV-1 DNA began at about 20 hr p.i. and remained at a similar level up to 48 hr (Table 1). The

TABLE 1. *Association of SV40 DNA with nuclear DNA extracted from CV-1 cells infected with SV40 in the presence of arabinosyl cytosine (Ara-C)*

Time after infection[a] (hr)	Counts/min of hybrid ^{3}H-labeled SV40 cRNA[b]		
	Supernatant DNA (Counts/min)	Sedimented DNA	
		(Counts/min)	(μg of DNA/filter)
0	1,428	21	98
10	1,301	58	91
20	665	442	87
30	154	579	103
48	45	643	89

[a] Time after adsorption with SV40 (0.5 PFU/cell) for over 2 hr. CV-1 cells infected with SV40 were seeded at a ratio of 1:2 and cultured in the presence of Ara-C.

[b] Nuclei (1×10^7 to 1.5×10^7) isolated from CV-1 cells at various times after infection were subjected to the selective extraction method of Hirt (6). From the supernatant and sedimented fractions, DNA was extracted and immobilized on the filter to hybridize with ^{3}H-labeled SV40 complementary RNA (cRNA) (1.37×10^5 counts/min). The value for normal CV-1 cell DNA (210 counts per min per 100 μg) was subtracted from the observed values.

total amount of free SV40 in the nuclei progressively decreased, as was the case with SV40-infected Chinese hamster cells (5), and as has been reported for monkey kidney cells by others (1). To determine whether the hybridizable DNA was covalently linked to cellular DNA, isolated nuclei were layered on alkaline sucrose gradients (10–30%) and centrifuged for 8 hr at 84,000 × *g* in a Beckman Spinco SW25.1 rotor by the method described previously (5). As shown in a reconstruction experiment of a mixture of ^{14}C-leucine- and ^{14}C-lysine-labeled CV-1 nuclei and ^{3}H-labeled SV40 DNA (Fig. 1), nuclear DNA was clearly separated from free SV40 DNA and protein. With the CV-1 cells at 6 hr p.i., most of the DNA hybridizable with

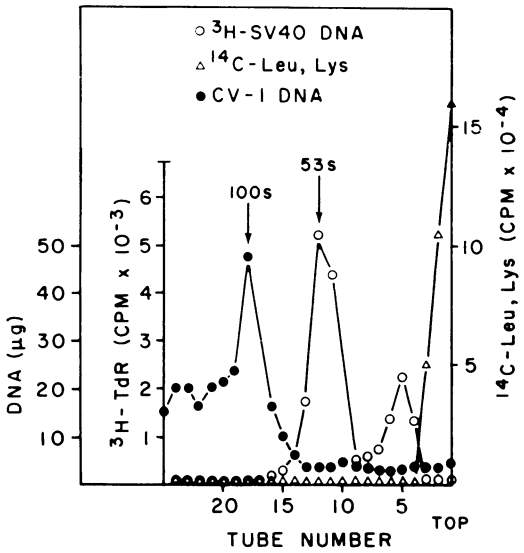

FIG. 1. *Size distribution of CV-1 DNA and ^{3}H-labeled SV40 DNA in an alkaline sucrose gradient. Confluent monolayers of CV-1 cells were split at a ratio of 1:2 and cultured in the presence of 0.1 μCi of ^{14}C-leucine and ^{14}C-lysine per ml for 48 hr and washed with phosphate-buffered saline. The labeled nuclei (1.8 × 10^{7}) were isolated by the method of Penman (9) and mixed with ^{3}H-labeled SV40 DNA (9 × 10^{4} counts/min; 1.55 × 10^{6} counts per min per μg) and layered overnight on alkaline sucrose (10–30%) in 0.3 N NaOH, 0.01 M ethylenediaminetetraacetate, 0.5 M NaCl in three centrifuge tubes of an SW25.1 rotor. They were then centrifuged for 8 hr at 84,000 × g. The fractions (1 ml/tube) were collected from the top of the gradient by an ISCO density gradient fractionator. Similar fractions from the three gradients were combined; 0.2 ml was precipitated with 5% trichloroacetic acid and counted in a liquid scintillation counter. The rest of the sample was also precipitated with 5% trichloroacetic acid and assayed for DNA by the diphenylamine method of Burton (2).*

^{3}H-labeled SV40 complementary RNA (cRNA) was present in the fractions representing free SV40 DNA (Fig. 2a). However, at 30 hr p.i., the majority of the hybridizable DNA was

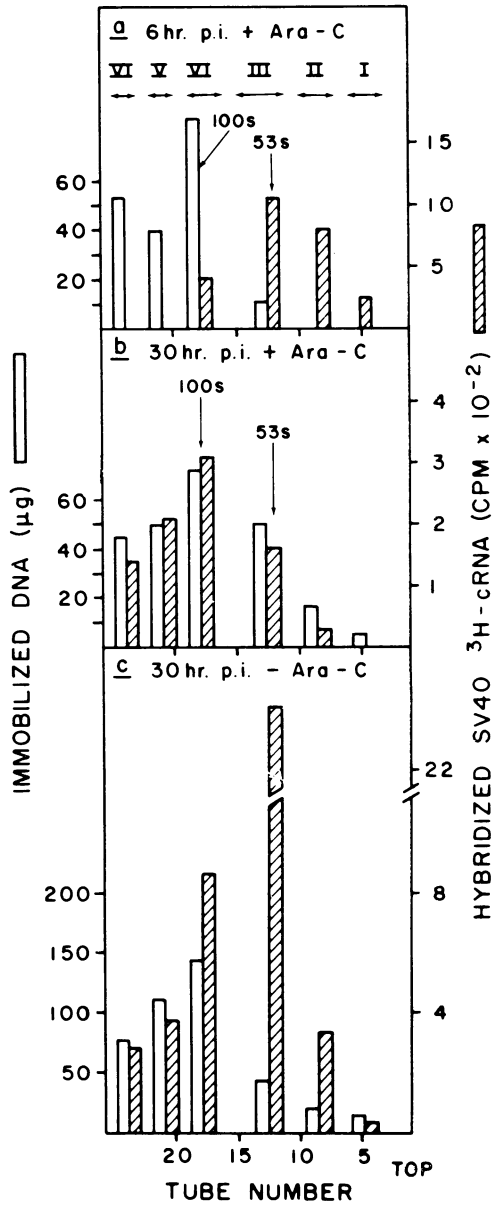

FIG. 2. *Size distribution of CV-1 DNA and SV40 DNA hybridized with ^{3}H-labeled SV40 complementary RNA (cRNA) in alkaline sucrose gradient. Confluent monolayers of CV-1 cells in 10 1-liter Blake bottles were infected with 0.5 PFU of SV40 per cell, split at a ratio of 1:2, and cultured in the presence of 15 μg of arabinosyl cytosine (Ara-C) per ml. At various times thereafter [(a) 6 hr p.i., plus Ara-C; (b) 30 hr*

associated with high-molecular-weight cellular DNA by alkali-stable linkages (Fig. 2b). When CV-1 cells were infected in the absence of Ara-C, a large fraction of the DNA hybridizable with SV40 cRNA was found in the fraction corresponding to SV40 DNA component 1 (53S) (Fig. 2c). However, the specific activity of the hybridizable DNA in the faster-sedimenting fractions (V and VI) was similar to that of Fig. 2b.

It is improbable that the hybridizable DNA found in the high-molecular-weight cell DNA represents oligomers of SV40 DNA since SV40 oligomers have been found only during replication of the viral DNA (7), and only monomeric DNA is incorporated in the virions (3). Under our experimental conditions no replication of the viral DNA can be detected (Table 1); furthermore, the oligomers are extracted in the supernatant fraction in the Hirt method (6). It is thus evident that the SV40 DNA integrates into the DNA of permissive, as well as nonpermissive, cells. Similar findings have been obtained in mouse cells infected by polyoma virus (R. K. Ralph and J. S. Colter, *in press*) and confirmed under a different set of conditions in another laboratory (Holzel and Sokol, *personal communication*).

p.i., plus Ara-C; (c) 30 hr p.i., minus Ara-C] nuclei were isolated. A total of 4 × 10⁶ to 6 × 10⁶ nuclei were layered on an alkaline sucrose gradient and centrifuged as described in the legend of Fig. 1. The fractions were pooled as indicated by Roman numerals and dialyzed against 0.1 × SSC (0.15 M NaCl, 0.015 M sodium citrate). NaOH was added to a final concentration of 0.3 M. After 10 min, the pH was adjusted to 7 with 12 N HCl. The concentration of SSC was adjusted to 6 × SSC. The samples were then immobilized on the membrane filter for hybridization with ³H-labeled SV40 cRNA (1.35 × 10⁵ counts/min). The amount of immobilized DNA on the filter was measured by the diphenylamine method after hybridization.

Because permissive cells can also be transformed by these DNA viruses under restrictive conditions (8), the significance of integration during the early phases of the replicative cycle of the virus cannot be clearly assessed at the present.

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