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Properties of Simian Virus 40 Rescued from Cell Lines Transformed by Ultraviolet-Irradiated Simian Virus 40

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Simian virus 40 (SV40) strains have been rescued from various clonal lines of mouse kidney cells that had been transformed by ultraviolet (UV)-irradiated SV40. To learn whether some of the rescued SV40 strains were mutants, monkey kidney (CV-1) cells were infected with the rescued virus strains at 37 C and at 41 C. The SV40 strains studied included strains rescued from transformed cell lines classified as "good," "average," "poor," and "rare" yielders on the basis of total virus yield, frequency of induction, and incidence of successful rescue trials. Four small plaque mutants isolated from "poor" yielder lines and fuzzy and small plaque strains isolated from an "average" and a "good" yielder line, respectively, were among the SV40 strains tested. Virus strains rescued from all classes of transformed cells were capable of inducing the transplantation antigen, and they induced the intranuclear SV40-T-antigen, thymidine kinase, deoxyribonucleic acid (DNA) polymerase, and cellular DNA synthesis at 37 C and at 41 C. With the exception of four small plaque strains rescued from "poor" yielders, the rescued SV40 strains replicated their DNA and formed infectious virus with kinetics similar to parental SV40 at either 37 or 41 C. The four exceptional strains did replicate at 37 C, but replication was very poor at 41 C. Thus, only a few of the rescued virus strains exhibited defective SV40 functions in CV-1 cells. All of the virus strains rescued from the "rare" yielder lines were similar to parental SV40. Several hypotheses consistent with the properties of the rescued virus strains are discussed, which may account for the significant variations in virus yield and frequency of induction of the transformed cell lines.

Of 83 cloned lines of mouse kidney cells (mKS-U) that were transformed at high input multiplicity by ultraviolet (UV)-irradiated SV40, 48 failed to yield simian virus 40 (SV40) by any rescue method so far employed. The remaining mKS-U lines have been classified as "rare," "poor," "average," and "good" yielders on the basis of (i) frequency of induction of SV40, (ii) size of SV40 yields, and (iii) the incidence of positive trials when the transformed cells were mixed with susceptible green monkey kidney (CV-1) cells in the presence of UV-irradiated Sendai virus (5). SV40 was recovered from "poor" yielders in less than 50% of the tests and from "rare" yielders on only one or two occasions in six or more trials, and in each case in very small amounts per culture. "Good" and "average" yielder lines yielded SV40 in all tests, and the frequencies of induction were $>10^{-4}$ and $>10^{-5}$, respectively.

Transformed clonal mKS-U lines classified as nonyielders probably contain SV40 genomes defective in functions essential for release from integration or virus replication. However, the reason why the transformed clonal lines which did yield SV40 varied so significantly in the frequency of induction and virus yield is unclear. It seemed possible that some of the "rare" or "poor" yielder lines might contain leaky SV40 mutants and that virus rescued from these lines might be defective in some biochemical function. Although virus rescued from only 2 out of 19 lines classified as "good" or "average" yielders had mutant plaque morphology, SV40 rescued from 4 out of 11 "poor" yielder lines were plaque morphology mutants. On the other hand, SV40 rescued from all five "rare" yielder lines had normal plaque morphology.

To learn whether some of the rescued SV40 strains were, in fact, defective in biochemical functions, the capacity of rescued virus strains to induce wild-type functions was studied after pro-

ductive infection of CV-1 cells at 37 C and, in some cases, at 41 C. The SV40 functions studied were induction of: (i) intranuclear T antigen; (ii) transplantation antigen; (iii) thymidine kinase and deoxyribonucleic acid (DNA) polymerase activities, and (iv) cellular DNA synthesis. In addition, the kinetics of infectious SV40 DNA and virus formation were studied as was the band centrifugation of ^{3}H-tritiated deoxythymidine (^{3}H-dT)-labeled SV40 DNA in CsCl density gradients. A preliminary report of the findings has been presented (S. Kit, T. Kurimura, and D. R. Dubbs, Bacteriol. Proc., p. 157, 1969).

MATERIALS AND METHODS

Cell lines. CV-1, an established line of green monkey kidney cells, and SV40-transformed mouse kidney cells (mKS) were grown in monolayer cultures as previously described (7, 10). All of the transformed cell lines were cloned prior to rescue experiments. The transformed cell lines contained the SV40 T antigen, but cell-free extracts failed to yield infectious SV40 when assayed on CV-1 monolayers.

Virus strains. Parental SV40 clone 307L and virus strains isolated from transformed mKS-U lines were assayed in monolayer cultures of CV-1 (10). SV40 strains recovered from transformed lines are designated SV40 (mKS-U3), SV40 (mKS-U4), etc. (5, 7). Most of the rescued SV40 strains were isolated from infectious centers produced either after plating mKS-U cells with CV-1 cells or after plating of heterokaryons of transformed and CV-1 cells during rescue (frequency of induction). Strain SV40 (mKS-U7 DNA) was initially recovered as infectious DNA in one of the rescue trials from mKS-U7. All prior and subsequent rescue trials for infectious DNA or virus with mKS-U7 were unsuccessful. Virus strains SV40 (mKS-U16), SV40 (mKS-U18), SV40 (mKS-U31), and SV40 (mKS-U65) were rescued from "rare" yielder cell lines. These four virus strains, as well as SV40 (mKS-U70), were isolated from plaques obtained during assay of virus yield from UV-Sendai treated mixtures of transformed and CV-1 cells. Two strains of virus were isolated from mKS-U1. Infectious centers produced by heterokaryons of mKS-U1, a "poor" yielder line, are usually small and indistinct (5). The virus isolated from these infectious centers was of the small plaque type and is designated SV40 (mKS-U1) sc. Occasionally, however, a large clear infectious center has been obtained from heterokaryons of mKS-U1 and CV-1 cells (5). A large plaque strain of virus, SV40 (mKS-U1) lc was obtained from such an infectious center. Except for SV40 (mKS-U1) sc and SV40 (mKS-U94) sc, all virus strains were used without further plaque purification. A pool of seed virus (passage 1, CV-1) was prepared from the plaques or infectious centers obtained during rescue. Stocks of virus strains to be used for biochemical experiments were then prepared from the seed virus (passage 2, CV-1).

Biochemical and enzyme experiments. The methods for assay of DNA polymerase and thymidine kinase have been described (8, 15). The induction of cellular DNA synthesis by SV40 and the formation of ^{3}H-dT-labeled SV40 DNA were determined by nitrocellulose column chromatography and band centrifugation in CsCl density gradients (9). In the same experiments, portions of the labeled cells were used for radioautographic studies (11). Infectious SV40 DNA was assayed by the method of Kit et al. (12).

Assay of SV40-T-antigen and transplantation antigen. The presence of SV40 tumor antigen in cell-free extracts of infected cells was demonstrated by complement fixation, with ascitic fluid from hamsters bearing SV40 transplant (virus-free) tumors and two full units of complement (7). The capacity of rescued SV40 strains to induce the transplantation antigen and the presence of the transplantation antigen in transformed cell lines were determined by immunorejection experiments, by using a transplantable mouse tumor line established in this laboratory (13).

RESULTS

Induction of thymidine kinase and DNA polymerase activities. During productive infection of green monkey kidney (CV-1) cells, parental SV40 clone 307L induces thymidine kinase and DNA polymerase activities (10, 15). SV40 strains rescued from the mKS-A, mKS-BU 25, and the mKS-BU 100 lines of transformed cells (all "good" yielders) induce these enzymes at 37 C, as well as other SV40 functions (7). To learn whether virus rescued from mKS-U clonal lines induced thymidine kinase and DNA polymerase activities, the experiments shown in Tables 1 and 2 were carried out. Confluent monolayer cultures of CV-1 cells were infected at input multiplicities ranging from 25 to 75 plaque-forming units (PFU)/cell and the infected cells were incubated for 43 or 48 hr at either 37 or 41 C (10). Enzyme induction at 41 C was studied to unmask possible temperature-sensitive mutants of the rescued virus strains.

Tables 1 and 2 show that thymidine kinase and DNA polymerase activities were induced at 37 C and at 41 C by SV40 strains rescued from "good," "average," "poor," or "rare" yielders. Among the virus strains tested were SV40 (mKS-U4), a fuzzy plaque mutant; SV40 (mKS-U1) lc, a large plaque strain; and SV40 (mKS-U1) sc, SV40 (mKS-U88), and SV40 (mKS-U94) sc, small plaque mutants.

In the preceding experiments, infected cells were harvested at 43 or 48 hr after infection. However, with SV40 (mKS-U6), enzyme inductions were also measured from 27 to 51 hr after infection. The time course of the enzyme inductions by SV40 (mKS-U6) was very similar to that by parental (clone 307L) virus.

Induction of intranuclear T antigen. Table 2 shows that virus strains rescued from "good," "average," "poor," and "rare" yielders induce the

SV40-specific intranuclear T-antigen at either 37 C or 41 C during productive infection of CV-1 cells.

Transplantation antigen. A transplantable tumor of inbred mice has recently been obtained by inoculating BALB/c mice subcutaneously with SV40-transformed mouse kidney (mKS-A) cells. Tissue culture lines (mKS-A TU-3, mKS-A TU-4, and mKS-A TU-5) derived from this tumor have been employed as challenge cells in immunorejection studies in mice preimmunized with SV40 clone 307L or SV40-transformed cell lines (13). These studies have demonstrated that tumor cells possess the SV40 transplantation antigen and that transformed cell lines mKS-U13 (a "good" yielder), mKS-U18 (a "rare" yielder), and mKS-U5, mKS-U7, or mKS-U17 (nonyielders) share a common transplantation antigen with the tumor cells. The studies have also shown that SV40 clone 307L can induce the transplantation antigen. In control experiments, it was shown that immunization two or three times with 10^6 primary mouse (BALB/c or Yale Swiss) kidney or embryo cells or with CV-1 cells did not prevent tumor formation by mKS-A TU-4 or TU-5 cells.

TABLE 1. *Induction of thymidine (dT) kinase activity by SV40 strains rescued from mKS-U lines at 43 hr after infection of CV-1 Cells at 37 or 41 C*

SV40 strain (rescued from mKS-U cells)	Classification of transformed cell	Activity[a] of dT kinase of cells grown at			
		37 C		41 C	
		Control	Infected	Control	Infected
Clone 307L		0.3	3.8	0.1	3.5
U10	Good	0.6	2.6	0.4	4.1
U13	Good	0.5	4.4		
U70	Good	0.6	5.1	0.3	3.5
U88 (sc)[b]	Good	0.6	7.0	0.4	6.7
U4	Avg	0.3	2.4	0.1	4.0
U6	Avg	0.6	4.8	0.5	4.4
U1 (sc)[b]	Poor	1.0[c]	4.2[c]	0.7[c]	2.4[c]
U94 (sc)[b]	Poor	1.0[c]	7.3[c]	0.7[c]	4.3[c]
U80	Poor	1.0	18.2	0.4	20.8
U65	Poor	0.6	3.8	0.3	4.1
U82	Poor	0.6	4.6	0.3	3.0
U18	Rare	0.3	8.1	0.1	3.9
U23	Rare	0.3	2.8	0.05	1.5

[a] Expressed as picomoles of deoxyuridine formed per microgram of protein in 10 min at 38 C.
[b] Small clear plaques (sc).
[c] Cells harvested 48 hr after infection.

Table 3 shows that small plaque mutant SV40 (mKS-U88), fuzzy plaque mutant SV40 (mKS-U4), and virus strains rescued from "rare" and "poor" yielder lines of transformed cells all are capable of inducing the SV40 transplantation antigen.

Radioautographic experiments. To learn whether virus strains rescued from mKS-U lines stimulated DNA synthesis in productively infected CV-1 cultures, radioautographic experiments (Table 4) were carried out. At 34 to 44 hr

TABLE 2. *Induction of T antigen and DNA polymerase by SV40 strains rescued from transformed mKS-U cell lines 43 hr after infection of CV-1 cells at 37 or 41 C*

SV40 strains (rescued from mKS-U cells)	Classification of transformed line	T antigen (CFU/mg protein)		DNA polymerase activity[a]			
		37 C	41 C	37 C		41 C	
				Control	Infected	Control	Infected
Clone 307L		380	300	0.3	1.9	0.6	2.0
U70	Good	330	720			0.2	1.0
U8	Good	320		0.3	1.3		
U88 (sc)[b]	Good	370	320	0.6	1.1	0.3	0.9
U4	Avg	130	440	0.3	1.0	0.6	1.5
U6	Avg	540	380	0.4	0.9	0.2	0.5
U80	Poor	610	1500			0.3	2.8
U65	Poor	400	650			0.3	1.8
U18	Rare	610	1070	0.3	2.4	0.6	2.2
U1 (lc)[b]	Poor	310	410	0.6	2.0	0.3	1.3
U1 (sc)[b]	Poor	120[c]	140[c]				
U94 (sc)[b]	Poor	180[c]	140[c]				

[a] Picomoles tritiated thymidine triphosphate incorporated per microgram of protein in 30 min at 38 C.
[b] Small clear (sc) and large clear (lc) plaques.
[c] Cells harvested 48 hr after infection.

TABLE 3. *Evidence that SV40 strains rescued from mKS-U cell lines induce the SV40-transplantation antigen*

Expt	BALB/c mice immunized with SV40 strain	Rescued from (yielder)	Immunization[a] procedure (PFU inoculated)	No. mKS-TU 5 (challenge) cells inoculated				
				5×10^5	5×10^4	5×10^3	5×10^2	0
1	Control (saline-glucose)			2/4[b]	4/5	2/4	0/4	0/4
	Clone 307L (G)[c]		4.0×10^7	0/4	0/4	0/4	0/4	0/4
	Clone 307L (G-S)[c]		1.6×10^5	0/4	0/4	0/4	0/4	0/4
	(mKS-U4) G	Avg	1.9×10^8	0/4	0/4	0/4	0/4	0/4
	(mKS-U4) G-S	Avg	3.0×10^6	0/4	0/4	0/3	0/4	0/4
	(mKS-U88) G	Good	9.0×10^7	0/4	0/4	0/4	0/4	0/4
	(mKS-U88) G-S	Good	8.5×10^4	0/4	0/4	0/4	0/4	0/3
2	Control (saline-glucose)			6/6	4/6	2/6	0/6	0/5
	(mKS-U18) G	Rare	1.1×10^8	0/4	0/4	0/4	0/4	0/3
	(mKS-U18) G-S	Rare	1.2×10^6	2/4	0/4	0/4	0/4	0/4
	(mKS-U31) G	Rare	3.0×10^8	0/4	0/4	0/4	0/4	0/3
	(mKS-U31) G-S	Rare	3.6×10^6	1/4	0/4	0/4	0/4	0/4
	(mKS-U94) G	Poor	1.9×10^7	0/4	0/4	0/4	0/4	0/4
	(mKS-U94) G-S	Poor	1.5×10^5	3/4	1/4	0/4	0/4	0/4
3	Control (saline-glucose)			6/6	4/6	2/5		0/5
	(mKS-U23) G	Rare	9.0×10^6	0/5	0/5	0/5		0/5
	(mKS-U16) G	Rare	1.5×10^8	0/5	0/5	0/5		0/4
	(mKS-U16) G-S	Rare	2.1×10^6	4/5	1/5	0/5		0/5

[a] For immunization, young adult mice were inoculated two times with the indicated virus dose with an interval of 7 days between inoculations. At 14 days later in experiment 1 and 15 days later in experiments 2 and 3, mice were challenged with mKS-A TU-5 cells.

[b] Number of mice bearing tumors/number of mice inoculated.

[c] G, virus partially purified by Genesolv-D treatment; G-S, virus partially purified by Genesolv-D treatment and centrifugation in sucrose density gradients (13).

TABLE 4. *Radioautographic determination of per cent nuclei labeled with ^{3}H-dT at 34 to 44 hr after infection of CV-1 cells with SV40 clone 307L or SV40 rescued from transformed mouse (mKS-U) cells*

Transformed line from which SV40 strain rescued	Yielder classification of transformed cells	Per cent nuclei labeled in cells incubated at	
		37 C	41 C
None (uninfected)		13.6	0.8
Clone 307L (wild type)		64.1	42.6
mKS-U6	Avg	44.2	26.0
None (uninfected)		10.8	2.5
mKS-U80	Poor	64.4	45.4
mKS-U18	Rare	50.7	40.8

after infection, only 11 to 14% of the nuclei of uninfected CV-1 cells were labeled by ^{3}H-dT at 37 C, and this number was reduced to 0.8 to 2.5% when the infections were carried out at 41 C. Parental SV40 and virus strains rescued from "average," "poor," and "rare" yielder lines of mKS-U cells markedly increased the per cent nuclei labeled at either 37 or 41 C.

Nitrocellulose chromatography and band centrifugation experiments. The radioautographic experiments show that the percentage of cells in the culture synthesizing DNA is increased after infection by rescued strains of SV40. Nitrocellulose chromatography and band centrifugation studies in CsCl density gradients were carried out to determine the proportion of radioactivity in cellular and viral DNA (9). Uninfected and SV40-infected cells were incubated at 37 or 41 C and pulse-labeled with ^{3}H-dT at 34 to 44 hr after infection. The synthesis of cellular DNA at 34 to 44 hr was greatly inhibited when uninfected cells were incubated at 41 C (Fig. 1). After infection by virus strains rescued from "rare," "poor," "average," or "good" yielders, cellular DNA synthesis was stimulated and this stimulation was especially pronounced at 41 C. SV40 strain (mKS-U7 DNA) also stimulated cellular DNA synthesis.

SV40 DNA was synthesized by cells infected at either temperature with all of the virus strains shown in Figure 1, although less ^{3}H-dT was incorporated into SV40 DNA at 41 C than at 37 C at 34 to 44 hr postinfection. Studies of infectious SV40 DNA synthesis suggest that SV40

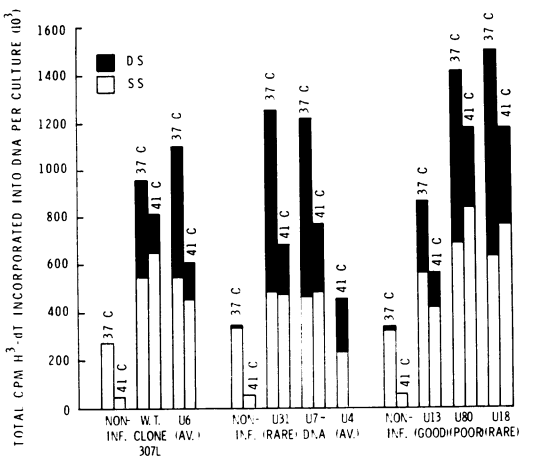

FIG. 1. *Estimation by nitrocellulose column chromatography of the relative amounts of SV40 DNA and cellular DNA synthesized by uninfected CV-1 cells and CV-1 cells infected with strains of SV40 rescued from mKS-U lines. DNA samples were heated at 100 C for 12 min prior to chromatography. Single-stranded (SS) DNA is the denatured DNA which requires 0.1 × SSC and 0.01 N NaOH for elution. Double-stranded (DS) DNA is that DNA which is not denatured by the heating and is eluted from the columns with 2 × SSC (circular SV40 DNA). Cells were labeled with ³H-dT (20 μc and 10 μg of dT per 20 ml of culture medium) from 34 to 44 hr after infection. Open bars represent denatured ³H-dT-labeled cellular (single-stranded) DNA, and black bars represent the ³H-dT-labeled SV40 (double-stranded) DNA. (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate.)*

DNA formation is initiated earlier at 41 C but may also slow down sooner.

Figures 2 and 3 depict band centrifugation experiments of ³H-dT-labeled DNA obtained from cells infected at 37 or 41 C with parental SV40 clone 307L or SV40 strains rescued from "average," "rare," and "poor" yielder lines of mKS-U cells. Similar results were obtained with SV40(mKS-U13), SV40(mKS-U4), SV40 (mKS-U31), and also with strain SV40 (mKS-U7 DNA). The radioactivity in fractions 1 to 21 represents cellular DNA and that of fractions 22 to 33 is due to SV40 DNA. Calculations based on the total ³H-dT incorporated into DNA in uninfected and infected cultures and the per cent of ³H-dT in cellular DNA (9) confirm that cellular DNA synthesis was stimulated in infected cultures at both 37 and 41 C. Fig. 2 and 3 also show that ³H-dT-labeled SV40 DNA from rescued virus strains banded at the same position as parental SV40 DNA. Since the nitrocellulose chromatography experiments indicate that heat-resistant DNA is made, the results suggest that the DNA of the rescued virus strains has the same conformation (circular) and about the same molecular weight as parental SV40 DNA.

Formation of infectious SV40 DNA and SV40 virus. A series of experiments were performed to learn whether the rescued virus strains were temperature-sensitive with respect to infectious DNA or SV40 virus formation (Fig. 4–7). These experiments were carried out either at relatively high input multiplicites, comparable to the preceding biochemical experiments, or at input multiplicities of 0.1 to about 3 PFU per cell. At the low input multiplicities, feasible because of the sensitivity of the infectious DNA assay, most

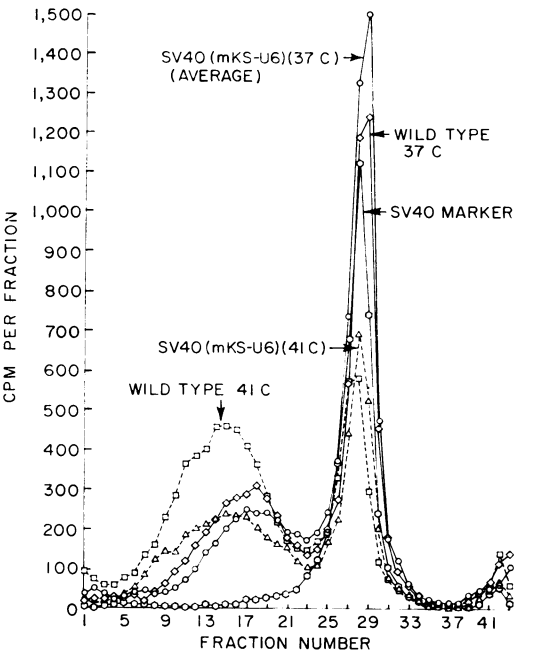

FIG. 2. *Sedimentation velocity (band centrifugation) of ³H-dT-labeled DNA from CV-1 cells infected with wild-type SV40 clone 307L and the SV40 (mKS-U6) strain rescued from an average yielder line. A marker SV40 DNA isolated from ³H-dT-labeled and purified parental SV40 particles was also studied. Cells were infected at 37 or 41 C and labeled with ³H-dT 34 to 44 hr after infection. The DNA preparations were centrifuged for 2.5 hr at 35,000 rev/min at 20 C in the SW 39 rotor of a Spinco model L2 ultracentrifuge. Bulk solution: 3 ml of CsCl; density 1.503 g/cm³. Lamella: 100 μliters of DNA. The solution was overlayed with 1.8 ml of paraffin oil (petrolatum). Eight drop fractions were collected on 2.5-cm squares of Whatman No. 4 paper, washed with 5% trichloroacetic acid and ethyl alcohol, dried for 1 hr at 70 C, and counted in a Packard Tri-Carb liquid scintillation spectrometer.*

of the CV-1 cells probably received only one infectious virus particle, so that any complementation was reduced. The results of these experiments show that parental SV40 clone 307L and virus strains rescued from "good" (Fig. 4), "average" (Fig. 5), and "rare" (Fig. 6) yielder lines of transformed cells are all capable of replicating at 37 and 41 C. In addition to the

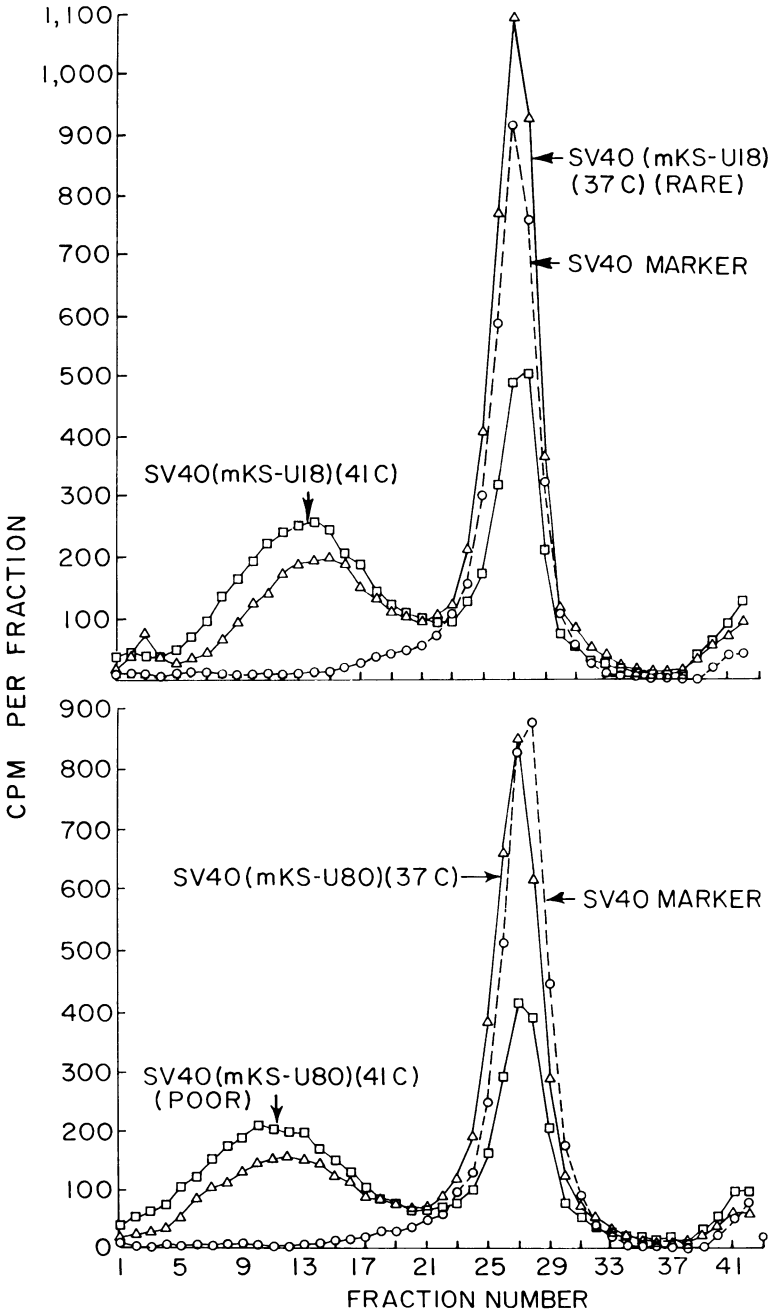

FIG. 3. *Sedimentation velocity (band centrifugation) of ^{3}H-dT labeled DNA from CV-1 cells infected with rescued virus strains, SV40 (mKS-U80) and SV40 (mKS-U18). Cells were infected at 37 or 41 C and pulse-labeled with ^{3}H-dT from 34 to 44 hr after infection. See legend to Fig. 2.*

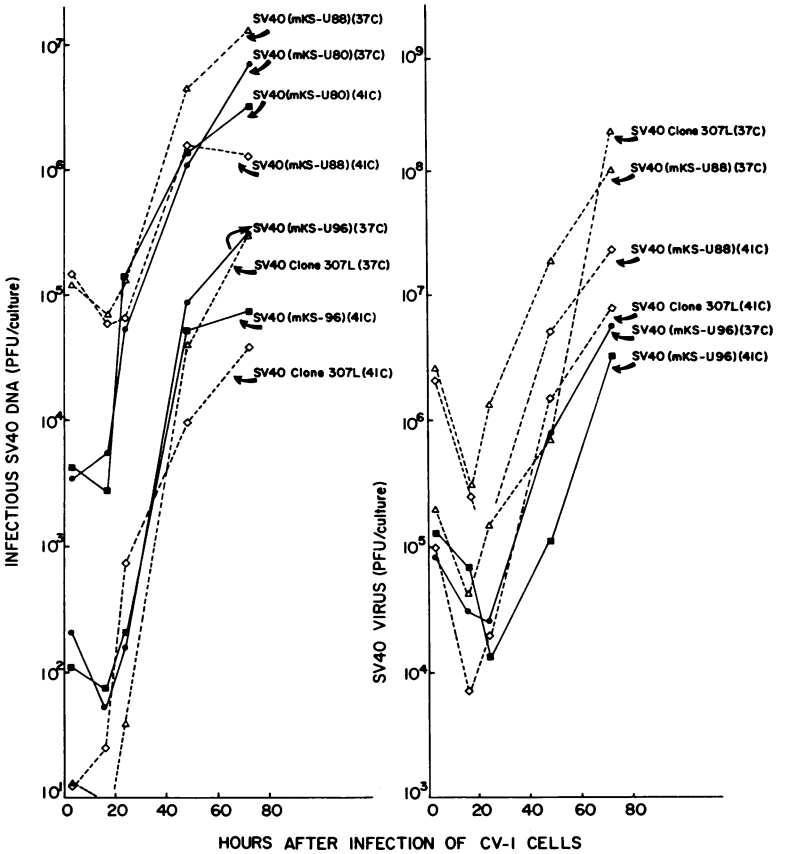

FIG. 4. *Kinetics of replication of infectious DNA and SV40 virus in CV-1 cells. Confluent monolayer cultures were infected at 37 or 41 C with parental SV40 clone 307L or strain SV40 (mKS-U96), rescued from a "good" yielder line, at an input multiplicity of 0.1 PFU/cell. The input multiplicities of SV40 (mKS-U88) small plaque mutant obtained from a "good" yielder, and SV40 (mKS-U80), a strain rescued from a "poor" yielder, were 6 and 1 PFU per cell, respectively. The replication curve for SV40 (mKS-U80), omitted from Fig. 4 to reduce crowding, was similar to that of parental virus.*

strains shown in the Fig. 4–6, SV40 (mKS-U10) and SV40 (mKS-U13), viruses rescued from "good" yielders, and SV40 (mKS-U16), rescued from a "rare" yielder, were studied with similar results. Usually, infectious DNA synthesis was initiated earlier at 41 C, but the final yields obtained at 72 to 96 hr after infection were lower at 41 C than at 37 C. Typical "crossover" curves are shown in Fig. 5.

The replication of SV40 (mKS-U80), a strain with parental SV40 plaque morphology rescued from a "poor" yielder, was also normal (Fig. 4). In contrast, four virus strains rescued from "poor" yielder lines mKS-U1, U3, U46, and U94, which form small plaques at 37 C, replicated poorly at 41 C. Experiments on the kinetics of infectious DNA and SV40 virus synthesis at 37 and 41 C by two of these strains, SV40 (mKS-U94) and SV40 (mKS-U46), are shown in Fig. 7.

DISCUSSION

Over 70 clonal lines of mouse kidney and 3T3 cells transformed by unirradiated SV40 in our laboratory have readily yielded virus when cocultivated with suceptible green monkey kidney (CV-1) cells, either with or without prior treatment with UV-Sendai (5, 7, 14; D. R. Dubbs and S. Kit, Bacteriol. Proc., p. 155, 1969). Of 83 clonal lines of mouse kidney (mKS-U) transformed by ultraviolet-irradiated SV40, 35 have also yielded virus after fusion with susceptible cells, but the incidence of successful rescues and the frequency of induction varied greatly among the mKS-U lines. Some of the mKS-U lines were "good" SV40 yielders, but 11 lines were "poor" yielders and 5 were "rare" yielders. At the extremes, rescue trials were always successful from "good" yielders but were successful only once or

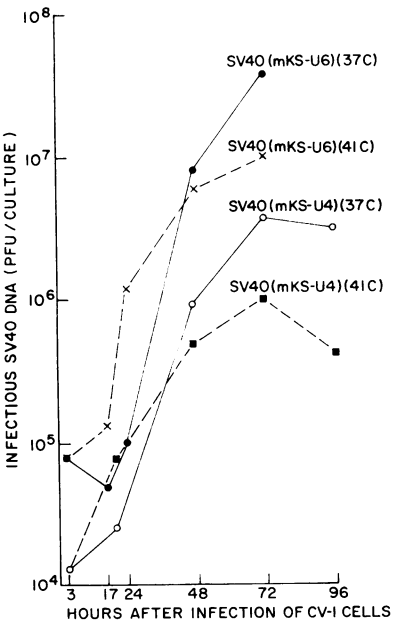

FIG. 5. *Kinetics of infectious DNA formation after infection of CV-1 cells at 37 or 41 C with virus strains rescued from "average" yielder lines of transformed cells. Input multiplicities were 4 PFU/cell for SV40 (mKS-U4), a fuzzy plaque mutant, and 20 PFU/cell for SV40 (mKS-U6), a strain showing parental virus plaque morphology.*

twice from "rare" yielders, and then with very low virus yields.

A series of studies have been carried out to elucidate the reasons for the heterogeneity of response of the mKS-U lines. It was shown that "rare" yielders and "good" yielders were equally capable of fusing with susceptible CV-1 cells (6). Thus, failure to fuse with susceptible cells does not account for the "rare" yielders. The effect on rescue of varying the input multiplicity during transformation was also considered (14). "Rare" yielders were not obtained when mouse kidney cultures were transformed with nonirradiated SV40 at input multiplicities ranging from 0.06 to 200 PFU per cell. On the contrary, "good" yielders were obtained even when mouse cultures were transformed at the low input multiplicity of 0.06 PFU/cell. Of transformed mouse kidney cells 30 clonal lines isolated from cultures infected at various input multiplicities with UV-irradiated SV40 (0.01 to 0.04 survival) were also studied. Of these, 22 were nonyielders, including all clonal lines transformed by UV-irradiated SV40 at input multiplicites of 0.004 and 0.06 PFU of total virus per cell (4×10^{-5} and 6×10^{-4} PFU of residual live virus per cell). However, two out of six clonal lines transformed at an input multiplicity of 50 PFU of total virus per cell (2 PFU of residual live virus per cell) were "rare" yielders, and four were nonyielders. Also, out of six clonal lines transformed at an input multiplicity of 0.6 PFU of total virus per cell (0.006 PFU of residual live virus per cell), one "rare," one "poor," and four nonyielders were found. Thus, "rare" yielders were obtained when mouse kidney cells were infected with an excess of UV-irradiated SV40 at intermediate input multiplicities. At very low input multiplicities of UV-irradiated SV40, only nonyielders were obtained.

In the present study, the properties of rescued virus strains were examined. It was found that rescued virus strains expressed all of the functions of parental SV40 during productive infection of CV-1 cells, whether rescued from "good," "average," "poor," or "rare" yielders, with the exception of four small plaque strains to be discussed later. Virus strains rescued from all classes of transformed cells were capable of inducing the transplantation antigen, and they induced the T-antigen, thymidine kinase, DNA polymerase, and cellular DNA synthesis at 37 and 41 C. With the exception of strains SV40 (mKS-U1) sc, SV40 (mKS-U3), SV40 (mKS-U46), and SV40 (mKS-U94), the rescued SV40 strains replicated their DNA and formed infectious virus with kinetics similar to parental SV40 at either 37 or 41 C. The four exceptional strains did replicate at 37 C, but replication was very poor at 41 C. This is not too surprising, since plaque formation was slow and small indistinct plaques were formed by the latter four strains even at 37 C. Thus, four small plaque strains rescued from "poor" yielders appear to have mutant properties, but other strains rescued from "poor" yielders and all of the strains rescued from "rare" yielders resembled parental SV40.

The finding that virus strains rescued from "rare" yielders have "normal" properties can be explained in two ways. (i) The integrated viral genome(s) may be normal, but the transformed cell may have properties which interfere with SV40 rescue, or (ii) the integrated viral genome(s) may, in fact, be defective. Transformed cell lines probably differ in genetic makeup and karyotype and, hence, in metabolic characteristics. "Rare" yielder lines might contain high levels of SV40 inhibitors (e.g., interferon or repressors), whereas the concentration of inhibitory substance may be relatively low in "good" yielders (1, 4, 17). "Excision" from the integrated state may entail the function of host cell enzymes, which might be deficient in "rare" yielder lines. In these instances, activation of SV40 replication would be rare, despite the integration of normal SV40

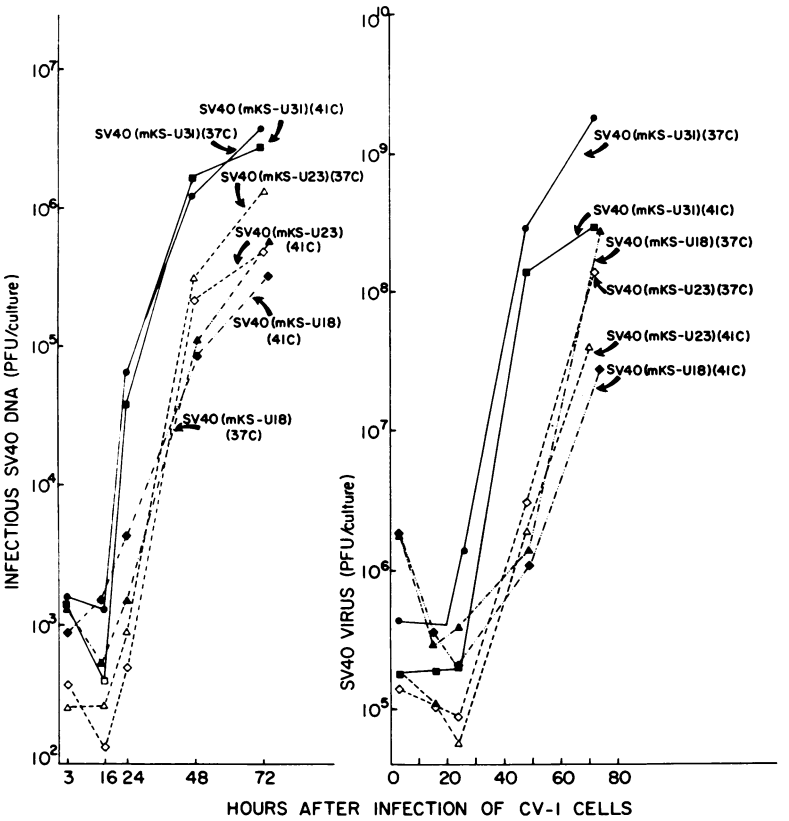

FIG. 6. *Kinetics of infectious DNA and SV40 virus formation after infection of CV-1 cells at 37 C or 41 C with SV40 strains rescued from "rare" yielder lines of transformed cells. The input multiplicities were 0.4 to 0.7 PFU per cell.*

genomes. Perhaps the observations by Burns and Black (2) that certain SV40-transformed hamster lines are inducible when treated with mitomycin C can be explained along these lines. Mitomytin C enhances nuclease activity in various cell lines. It is one among several agents possessing anti-interferon activity which enhances virus yield and plaque size (3, 17).

If defective SV40 genomes were integrated, pseudo-wild-type virus strains may, nevertheless, have been rescued for the following reasons.

(i) Two or more defective genomes might be integrated in the "rare" yielder lines of mKS-U cells. Occasional recombination or effective complementation after activation of SV40 could have occurred. Serial passage would result in a selection for virus possessing pseudo-wild-type properties. The double lysogen-recombination hypothesis gains credence from recent studies from this laboratory demonstrating that double lysogens may be generated by transforming 3T3 cells with small plaque strain SV40 (mKS-U88) and fuzzy plaque strain SV40 (mKS-U4) (D. R. Dubbs, and S. Kit, Bacteriol. Proc., p. 155, 1969). From some of the clonal lines of transformed cells, both small and fuzzy plaque type viruses have been rescued. In addition, clonal lines were obtained from which small, fuzzy, and large clear (wild-type) plaque formers were recovered. In the initial experiments in which UV-irradiated SV40 was used to transform mouse kidney cells to mKS-U lines, the input multiplicities were 30 and 186 PFU per cell (unirradiated SV40), so that some of the mKS-U lines could have been double lysogens (5).

(ii) Viral genomes integrated in "rare" yielder mKS-U lines may have contained lesions in genes essential for release of SV40 from the integrated state and, perhaps, in genes essential for integration, but not in those genes required for productive infection of CV-1 cells. This hypothesis presupposes that one type of SV40 gene corresponds to the "Int" gene of phage λ. Experiments showing that virus strains rescued from "rare" yielders are less able to transform mouse kidney

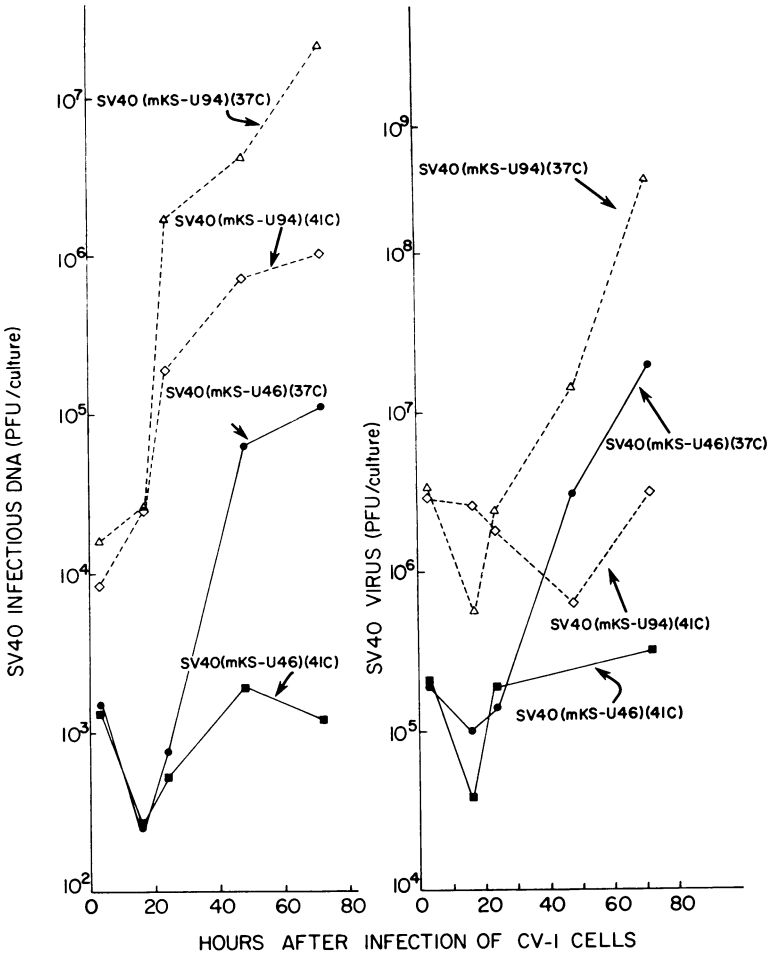

FIG. 7. *Kinetics of infectious DNA and SV40 virus formation after infection of CV-1 cells at 37 C or 41 C by small plaque mutants rescued from "poor" yielder lines of transformed cells. The input multiplicity for SV40 (mKS-U94) was 1 PFU/cell and that for SV40 (mKS-U46) was 0.8 PFU/cell.*

cells than those isolated from "good" yielders would support this hypothesis.

(iii) Reversion of UV-induced lesions in the integrated SV40 genome or reversion of mutations during serial passage of rescued virus strains might occasionally lead to the rescue of pseudo-wild-type virus from "rare" yielders. In connection with this last hypothesis, an effort was made to enhance possible reversions by cultivating "rare" yielder and nonyielder mKS-U lines in the presence of 5-bromodeoxyuridine for 60 to 100 passages. However, this treatment did not lead to rescue of SV40 from the "rare" or nonyielder mKS-U lines.

It is to be emphasized that the rescued virus strains replicated through several cycles prior to the biochemical testing. After the formation of plaques during rescue, seed virus was prepared from the plaques and then virus stocks were grown in CV-1 cells from the seed virus. Thus, there was ample opportunity for selection of pseudo-wild-type virus from a heterogeneous population of rescued particles. It is quite possible that the viruses formed during the first cycle of replication in the heterokaryons actually contained many mutant particles, which were not detected. However, since rescue experiments were performed with cloned lines of transformed cells, heterogeneity in the rescued virus population would have arisen from abnormal "excision" events, or, from heterogeneity of the integrated genomes in double and multiple lysogens, and not from heterogeneity of the population of transformed cells. Further electron microscopic and biophysical experiments are indicated on heterokaryons during the first 24 to 72 hr after fusion.

Such studies might reveal profound differences in the DNA and virus particles synthesized during the first cycle after fusion of various mKS-U lines and susceptible cells.

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LITERATURE CITED

1. Brailovsky, C. A., L. D. Berman, and Ch. Chany. 1969. Decreased interferon sensitivity and production in cells transformed by SV40 and other oncogenic agents. Int. J. Cancer 4:194–203.
2. Burns, W. H., and P. H. Black. 1968. Analysis of simian virus 40-induced transformation of hamster kidney tissue in vitro. V. Variability of virus recovery from cell clones inducible with mitomycin C and cell fusion. J. Virology 2:606–609.
3. Cantell, K. 1968. Enhancement of animal virus growth by another virus, p. 31–44. *In* M. Sanders and E. H. Lennette (ed.), Medical and applied virology. Warren H. Green, Inc., St. Louis.
4. Cassingena, R., and P. Tournier. 1968. Misé en évidence d'un "represseur" spécifique dans des cellules d'espèces différentes transformées par le virus SV40. Compt. Rend. Series D. 267:2251–2254.
5. Dubbs, D. R., and S. Kit. 1968. Isolation of defective lysogens from simian virus 40-transformed mouse kidney cultures. J. Virol. 2:1272–1282.
6. Dubbs, D. R., and S. Kit. 1969. Heterokaryon formation of simian virus 40-transformed cells in the presence of ultraviolet-irradiated Sendai virus. J. Virol. 3:536–538.
7. Dubbs, D. R., S. Kit, R. A. de Torres, and M. Anken. 1967. Virogenic properties of bromodeoxyuridine-sensitive and bromodeoxyuridine-resistant simian virus 40-transformed mouse kidney cells. J. Virol. 1:968–979.
8. Kit, S., and D. R. Dubbs. 1965. Properties of deoxythymidine kinase partially purified from noninfected and virus-infected mouse fibroblast cells. Virology 26:16–27.
9. Kit, S., R. A. de Torres, D. R. Dubbs, and M. L. Salvi. 1967. Induction of cellular deoxyribonucleic acid synthesis by simian virus 40. J. Virol. 1:738–746.
10. Kit, S., D. R. Dubbs, P. M. Frearson, and J. L. Melnick. 1966. Enzyme induction in SV40-infected green monkey kidney cultures. Virology 29:69–83.
11. Kit, S., D. R. Dubbs, and T. C. Hsu. 1963. Biochemistry of vaccinia-infected mouse fibroblasts (strain L-M). III. Radioautographic and biochemical studies of thymidine-^{3}H uptake into DNA of L-M and rabbit cells in primary culture. Virology 19:13–22.
12. Kit, S., T. Kurimura, M. L. Salvi, and D. R. Dubbs. 1968. Activation of infectious SV40 DNA synthesis in transformed cells. Proc. Nat. Acad. Sci. U.S.A. 60:1239–1246.
13. Kit, S., T. Kurimura, and D. R. Dubbs. 1969. Transplantable mouse tumor line induced by injection of SV40-transformed mouse kidney cells. Int. J. Cancer 4:384–392.
14. Kit, S., and M. Brown. 1969. Rescue of simian virus 40 from cell lines transformed at high and at low input multiplicities by unirradiated or ultraviolet-irradiated virus. J. Virol. 4:226–230.
15. Kit,S., L. J. Piekarski, and D. R. Dubbs. 1967. DNA Polymerase indcued by simian virus 40. J. Gen. Virology 1:163–175.
16. Rapp, F., and D. Vanderslice. 1965. Enhancement of the replication of measles virus by mitomycin C. J. Immunology 95:753–758.
17. Takemoto, K. K., G. J. Todaro, and K. Habel. 1968. Recovery of SV40 virus with genetic markers of original inducing virus from SV40-transformed mouse cells. Virology 35:1–8.