JOURNAL OF VIROLOGY, Nov. 1976, p. 391-399

Vol. 20, No. 2
Printed in U.S.A.

Simian Virus 40-Permissive Cell Interactions: Selection and Characterization of Spontaneously Arising Monkey Cells That Are Resistant to Simian Virus 40 Infection

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Received for publication 28 June 1976

A fraction of permissive cells survive simian virus 40 (SV40) infection. The frequency of such surviving cells depends only upon the concentration of infecting virus, both parental and progeny, to which the cells are exposed during the course of selection. Surviving clones, which can be freed of virus by cloning in the presence of SV40 antiserum, are indistinguishable from parental cells in their growth characteristics and display no SV40 T antigen; thus they are not transformed. Most surviving clones are less than 10% as susceptible as parental cells to SV40 infection; 5 to 10% are less than 1% as susceptible. None of these SV40-resistant clones is absolutely resistant to SV40 infection. Analysis of 16 independently arising resistant clones indicates that they all block SV40 infection at an early stage after adsorption and eclipse but before full uncoating. Viral mutants have been isolated that partially overcome the block to infection in these cells; these host range viruses plaque on resistant lines fivefold more efficiently than wild-type SV40 and have a characteristic plaque morphology. Fluctuation analysis indicates that resistant cells arise spontaneously during the growth of normally susceptible permissive cells. Thus, SV40-resistant cells are selected for, not induced by, SV40 infection.

Simian virus 40 (SV40) infection of African green monkey kidney cells causes a productive or lytic infection in which the infected cells die and liberate a large number of progeny virus (3, 24). Since SV40 contains only enough DNA to code for about 200,000 daltons of protein, many essential steps in its infection must be performed by host-coded products. Understanding the interplay of host and viral products is essential for understanding the precise mechanism of SV40 infection. To help define the relevant host contribution to the SV40 infectious process, we have begun to isolate from permissive cell populations those cells in which SV40 infection is blocked (SV40-resistant permissive cells). SV40 infection of such cell lines should be interrupted at a characteristic point that is dependent upon the altered host function. Physiological and biochemical analysis of these host-introduced blocks should help to elucidate the mechanism of SV40 infection in much the same way as does the complementary analysis of infections by mutant virus.

An analogous approach in bacteria-bacteriophage systems has yielded several bacterial mutants that no longer permit productive bacteriophage infection. The earliest studied of these bacterial mutants were ones involving alterations in the cell wall that prevented bacteriophage adsorption (5, 28). More recently, bacterial mutants have been isolated that block bacteriophage growth at intracellular stages such as DNA replication (2, 4, 6, 11, 26), transcription (9, 10), and assembly (12, 26). Other studies indicate that bacteria can prevent adequate translation of bacteriophage mRNA's by utilizing a set of tRNA's that is sufficient for its own needs yet unacceptable for an infecting bacteriophage (29).

In this paper we examine the most straightforward method for selecting SV40-resistant permissive cells; the isolation of cells that have survived exposure to SV40. Such surviving cells are partially resistant to SV40 infection because they block infection at an early stage, after adsorption and eclipse but before full uncoating. The block to infection in these cells probably involves a step that requires an interaction between host and viral gene products, since mutant virus can be isolated that partially overcomes the block. In addition, fluctuation analysis indicates that SV40-resistant cells

arise spontaneously within the predominantly susceptible population and are not induced by SV40 infection.

MATERIALS AND METHODS

Cells. Two established cell lines of African green monkey kidney origin were used: MA134, obtained from J. S. Pagano, for virus stock preparation, and CV1, obtained from S. Kit, for production of resistant lines and for plaque assays. Cells were grown in Dulbecco modified Eagle medium (GIBCO) supplemented with 10% calf serum (Microbiological Associates or GIBCO), penicillin (GIBCO), 100 units/ml, streptomycin (GIBCO), 100 μg/ml, and amphotericin B (GIBCO), 0.25 μg/ml. Cells were trypsinized with 0.05% trypsin (Nutritional Biochemicals) plus 2.5 mM EDTA in TS buffer (25 mM Tris, pH 7.4, 140 mM NaCl, 5 mM KCl, 0.7 mM K_2HPO_4) and were passaged routinely once every 7 days at a 1:10 or 1:20 dilution.

Virus. Plaque-purified SV40, strain Rh911 (13), was obtained from J. Vinograd and was used in all experiments. High-titer viral stocks were prepared by a modification of method IV of Estes et al. (7) after low multiplicity infection of MA134 cells (0.005 SV40 PFU per cell). SV40 titers were determined by a plaque assay using freshly confluent CV1 cells in petri plates (60 by 15 mm) (19).

Cell infections. Cell infections routinely were carried out for 1 h in a CO_2 incubator at 37°C using 0.2-ml (on 60- by 15-mm petri plates, Falcon) or 0.6-ml (on 90- by 15-mm petri plates, Nunc) portions of virus in TD buffer (TS buffer plus 0.5 mM $MgCl_2$ and 1.0 mM $CaCl_2$) plus 5% calf serum. Plates were shaken at 0 and 30 min. SV40 DNA infections were carried out for 15 to 20 min by a modification of the method of McCutchen and Pagano using DEAE-dextran (Pharmacia Fine chemicals) at 500 μg/ml (18). Only freshly confluent or slightly subconfluent cell monolayers were used for DNA infection, since confluent cells become increasingly less susceptible to DNA infection with age (J. Wilson, unpublished observations).

Cloning procedures. Clones were obtained by three different methods: (i) trypsinization of well isolated clones (with a cloning ring) from petri plates seeded at low cell density; (ii) distribution of appropriately diluted cells in a microtiter test plate (Linbro) as described by Robb and Martin (23); (iii) end-point serial dilution in a microtiter test plate, followed by visual inspection for wells that contained a single focus of growth.

SV40 antiserum. Horse SV40 antiserum (Flow) inactivated SV40 with a first order rate constant of 5 × 10^3/min at 37°C. Dulbecco modified Eagle medium containing 1% SV40 antiserum inactivated 2 × 10^7 SV40 PFU/ml in an extended incubation at 37°C. In experiments in which neutralization of extra-cellular virus was required, antiserum was added in 5- to 10-fold excess over the expected quantity of virus.

Standard method for isolation of resistant cells. Confluent monolayers of CV1 cells were infected for 1 h at a multiplicity of about 20 SV40 PFU per cell and replated 24 h later at 10^6 to 5 × 10^6 cells per 90-mm plate. Medium was changed on days 4 and 8 after infection. After 3 weeks individual clones were isolated. Cultures grown from surviving clones released virus into the medium and contained approximately equal numbers of infective centers and T antigen-positive cells. All these signs of SV40 infection were eliminated by cloning in the presence of SV40 antiserum. In general, isolated clones were recloned immediately by end-point dilution in a microtiter test plate in the presence of 1% SV40 antiserum.

T-antigen assay. T antigen was assayed by an indirect fluorescent-antibody technique, using hamster SV40 T-antiserum (Flow) and fluorescein-conjugated rabbit anti-hamster gamma globulin (Colorado Serum Co.) by the method of Pope and Rowe (20). From 200 to 400 cells were scored for each measurement.

Fluctuation analysis. Clones of CV1 cells were grown from one or a few cells to about 5 × 10^7 cells before testing. Fresh clones were prepared for each separate experiment to ensure as nearly as possible that each clone had undergone the same number of doublings. Confluent monolayers of the clones were infected at a multiplicity of about 20 SV40 PFU per cell. Twenty-four hours after infection the cells were counted in a hemacytometer and replated at a uniform cell density. In all fluctuation experiments the stated multiplicity of infection was calculated as the number of SV40 PFU added to a plate divided by the number of cells on that plate at the time of replating. Medium was changed on days 4 and 8 after infection. Surviving clones were stained with hematoxylin and counted at 3 weeks postinfection. Data from these experiments were subjected to fluctuation analysis (17) using the F test for equality of variances (15).

Isolation of SV40 host range virus. CV1 cells infected with SV40 wild-type virus were mutagenized with nitrosoguanidine as described by Tegtmeyer et al. (27). Mutagenized virus were grown at a multiplicity of 0.1 SV40 PFU per cell in CV1 cells, and portions were then cycled four times through either CV1e, CV1f, or CV1n cells. These virus were then plaqued on the resistant line in which they were grown and large, sharp-edged plaques were picked and plaque purified once.

RESULTS

Parameters that influence the fraction of permissive cells that survive an SV40 infection. Infection of a monolayer of CV1 cells with a multiplicity of greater than one SV40 PFU per cell causes extensive cell death in 3 to 4 days. However, after 2 weeks, surviving clones become visible. The parameters that influence the frequency of surviving clones were determined by examining four experimental variables (see Materials and Methods): (i) initial multiplicity of infection, (ii) secondary infection by progeny virus, (iii) density of cells after replating, and (iv) time after infection at which cells were replated.

The effects of initial and secondary infection

are shown in Fig. 1. Twenty-four hours after infection at the indicated multiplicities, cells were replated in the presence or absence of excess SV40 antiserum. The frequency of survivors decreased monotonically with increasing multiplicity of infection in both cases. Thus, the initial multiplicity is an important factor in determining survivor frequency. In addition, the 10- to 100-fold lower frequency in the absence of SV40 antiserum suggests that secondary infection by progeny virus also influences the survivor frequency. The effects of cell density and time of replating are shown in Fig. 2. As the density at which infected cells were replated increased, thereby increasing the concentration of progeny virus, the frequency of surviving clones decreased. Figure 2 also shows that there was no variation in the frequency of surviving clones until 16 to 20 h after infection, the time at which progeny viruses begin to be produced.

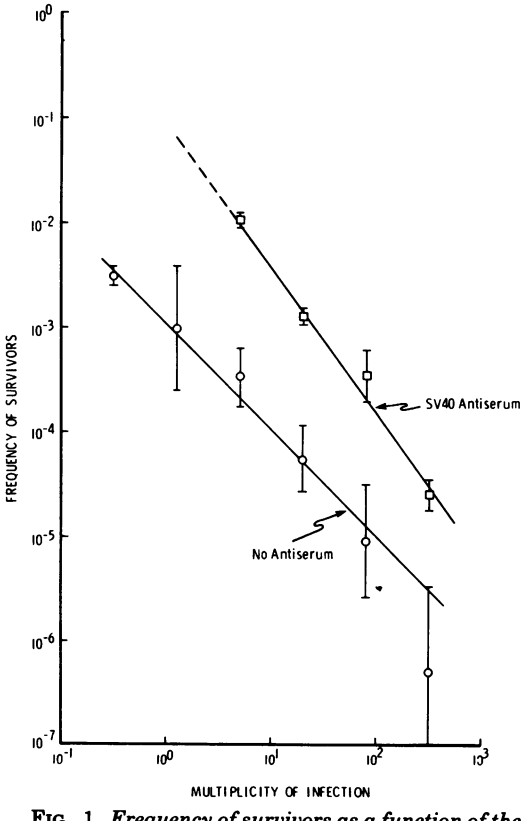

FIG. 1. *Frequency of survivors as a function of the multiplicity of infection. Confluent monolayers of CV1 cells were infected at the indicated multiplicities and replated 24 h after infection at 8 × 10⁴ cells/90-mm plate in the presence (□) or absence (○) of 4% SV40 antiserum. Medium was changed on days 4 and 8 after infection. Surviving clones were stained with hematoxylin 21 days after infection.*

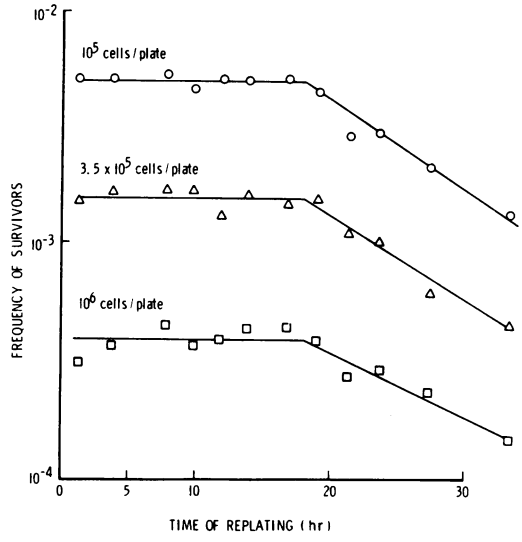

FIG. 2. *Frequency of survivors as a function of cell density and of the time of replating. Confluent monolayers of CV1-13 cells (a clone of CV1) were infected at a multiplicity of 5 SV40 PFU per cell and replated at the indicated times at 10⁶ cells/90-mm plate (□), 3.5 × 10⁵ cells/90-mm plate (△), and 10⁵ cells/90-mm plate (○). Medium was changed on days 4 and 8 after infection. Surviving clones were stained with hematoxylin 21 days after infection.*

These experiments are consistent with the simple interpretation that the frequency of surviving clones depends solely upon the concentration of infecting SV40, both parental and progeny, to which the cells are exposed during the course of the experiment.

Initial characterization of clones that survived an SV40 infection. Seventy-six surviving clones were isolated by the standard method from the parental CV1 line and from 37 primary and secondary subclones of CV1. Each was shown to be virus free and then was tested for resistance to SV40 by measuring yield of virus after a low-multiplicity infection (Fig. 3). Approximately 75% of the surviving clones yielded less than 10% the number of virus that the parental CV1 line yielded. Approximately 5 to 10% of the surviving clones yielded less than 1% the control amount. None of the surviving clones is absolutely resistant to SV40 infection. Cells from two surviving clones (CV1e and CV1f, see Table 1) were converted quantitatively to infective centers at multiplicities of infection greater than 100 SV40 PFU per cell. Thus, the partial resistance of surviving clones apparently is a uniform property of the cells and does not result from a mixed population of sensitive and absolutely resistant cells. The resistant state of surviving clones appears to be heritable, since they were grown from a single

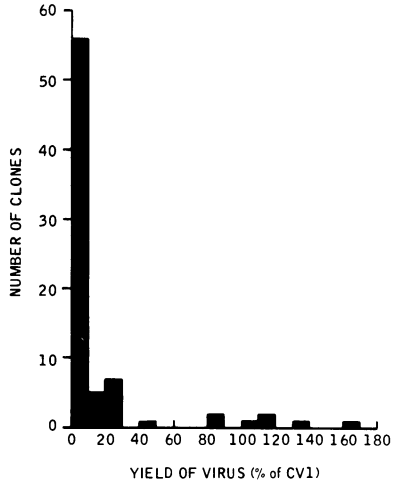

FIG. 3. *Sensitivity of surviving clones to SV40 infection. Freshly confluent monolayers were infected for 1 h with 10^4 SV40 PFU per 60-mm plate (a multiplicity of about 0.005 SV40 PFU per cell). At 48 h post-infection all cells were frozen. Subsequently, they were thawed, sonicated, and titered by plaque assay. Virus yields are expressed as percentage of a CV1 control.*

cell to a population of at least 10^7 cells (about 23 generations) in the absence of SV40 before testing.

Surviving cells that have been cloned in the presence of SV40 antiserum lack T antigen. In addition, growth curves of several resistant clones (CV1e, CV1f, CV1n, see Table 1) indicate that they grow at approximately the same rate and to the same cell density as CV1. By visual inspection, all surviving lines are contact inhibited. Finally, CV1 and two surviving lines (CV1e, CV1n) contain no detectable SV40 DNA (less than 0.1 genome of SV40 per cell) as measured by DNA:DNA reassociation kinetics. These observations taken together indicate that resistant cells isolated by our standard method are not transformed.

Partial analysis of the block to SV40 infection. Sixteen of the surviving clones shown in Fig. 3 were examined for progeny virus yield and T-antigen production after SV40 infection, for progeny virus yield after SV40 DNA infection, and adsorption of SV40 virus (Table 1). These cells are resistant to viral infection, as indicated by reduced progeny virus yields and T-antigen production, but are fully sensitive to DNA infection, as indicated by normal progeny virus yields. Thus, these cells are blocked at an early stage of infection before full uncoating. As shown in Table 1, all resistant cells adsorb SV40 normally by the operational definition of adsorption; i.e., ability to bind virus. More detailed studies on CV1f demonstrated that it removed virus from the medium with a first order rate constant of 1.8×10^{-2} min, 75% of the rate constant for CV1 in a parallel experiment. In addition, adsorbed SV40 enter the eclipse phase in normal and resistant cells, CV1e, CV1f, and CV1n, at approximately the same rate (70 to 90% eclipse within 2 h). Thus, these spontaneously arising resistant cells all seem to block SV40 infection at an early stage after adsorption and eclipse but before full uncoating.

TABLE 1. *Characterization of surviving clones*

Resistant clone	Virus infection		DNA infection (virus yield)[c]	Adsorption[d]
	Virus yield[a]	T antigen[b]		
CV1e	4.8	5.0	157	70
CV1f	0.8	1.4	61	140
CV1n	0.4	0.7	86	126
CV1-4a	1.1	5.0	107	95
CV1-13a	9.0	35	123	81
CV1-11-3d	0.6	8.0	81	75
CV1-11-4a	22.0	25.0	189	145
CV1-11-6a	16.0	24.0	64	57
CV1-11-11b	9.0	8.0	82	215
CV1-11-11c	66	41	250	223
CV1-11-11d	48	10.0	130	95
CV1-11-12d	8.4	21.0	330	100
CV1-11-14c	8.0	25.0	73	107
CV1-11-18c	1.0	3.0	149	107
CV1-11-19c	6.2	8.0	107	66
CV1-11-21a	35	22.0	88	144

[a] The yield of SV40 was determined 48 h after infection with 10^4 SV40 PFU per monolayer (about 2×10^6 cells). Cells were scraped into the medium and sonicated before plaque assay. Titers are the average of two experiments and are expressed as percentage of a CV1 control (2.5×10^5 PFU/ml).

[b] The fraction of cells expressing T antigen was measured 36 h after infection at a multiplicity of 2 SV40 PFU per cell and is expressed as percentage of a CV1 control (70% show T antigen).

[c] The yield of SV40 virus was determined 48 h after infection with 0.01 μg of SV40 DNA (about 10^4 PFU) per monolayer (about 2×10^6 cells). Cells were scraped into the medium and sonicated before plaque assay. Titers are the average of two experiments and are expressed as percentage of a CV1 control (3.3×10^5 PFU/ml).

[d] SV40 virus in TS buffer containing 5% calf serum was incubated with a monolayer of cells at 23°C at a multiplicity of 0.5 PFU per cell. After 5 min of incubation, cells were rinsed three times with TS buffer and quick frozen in liquid nitrogen. Cells were thawed and sonicated before plaque assay. Titers of cell-associated SV40 are expressed as percentage of a CV1 control (8.5×10^4 PFU/ml).

Characterization of SV40 host range viruses that grow more efficiently on resistant cells. Wild-type SV40 viruses make very small,

fuzzy-edged plaques on resistant lines, CV1e, CV1f, and CV1n, with an efficiency of plaque formation less than 1% of that on CV1. SV40s that make large, sharp-edged plaques on these resistant lines were isolated as described in Materials and Methods. A comparison of the plaques made by wild-type and host range viruses on resistant line CV1f is shown in Fig. 4. Wild-type and host range viruses make identical plaques on CV1 cells. The plaquing efficiency on resistant cells of host range viruses relative to wild type is shown in Table 2. Two points can be made from these data. (i) These SV40 host range viruses all plaque about fivefold more efficiently than wild-type SV40 on resistant lines, suggesting that they carry a mutation that partially compensates for the defect in the resistant cells; and (ii) a host range virus that was selected in one resistant cell line grows equally well on the other two cell lines tested, suggesting that the block to SV40 infection in all three resistant lines may be the same.

In addition, wild-type and host range DNA have equal plaquing efficiency (2×10^6 to 3×10^6 PFU/μg) on both normal and resistant cells, suggesting that the compensating mutation in the host range virus affects a structural component of the virus. It should be noted that plaques that form on the resistant cells have the morphology that is characteristic of the infecting genome. Equal efficiency of plaque formation on the resistant cells may seem surprising since only the first cycle of infection is by DNA. Apparently, the virus released from the initially infected cell is sufficient in number to overcome the block in surrounding cells and propagate the plaque. Consistent with this interpretation is the observation that CV1 cells infected with wild-type virus form plaques in an infective center assay with equal efficiency on normal and resistant cells. A detailed characterization of these host range viruses will be the subject of a subsequent communication.

Origin of SV40-resistant cells. The possible mechanisms by which resistant cells might arise fall into three general categories. (i) A

TABLE 2. *Plaquing efficiency of host range SV40 on resistant cells*[a]

Resistant clone	$SV40hr_{CV1e}$	$SV40hr_{CV1f}$	$SV40hr_{CV1n}$
CV1e	4.9[a]	4.3	4.8
CV1f	4.3	4.3	5.2
CV1n	5.2	4.5	6.2

[a] Plaquing efficiency of host range virus relative to wild-type SV40 (1.0) was calculated as follows: ($SV40hr_{CV1e}$ titer on CV1e/$SV40hr_{CV1e}$ titer on CV1)/(SV40 wild-type titer on CV1e/SV40 wild-type titer on CV1).

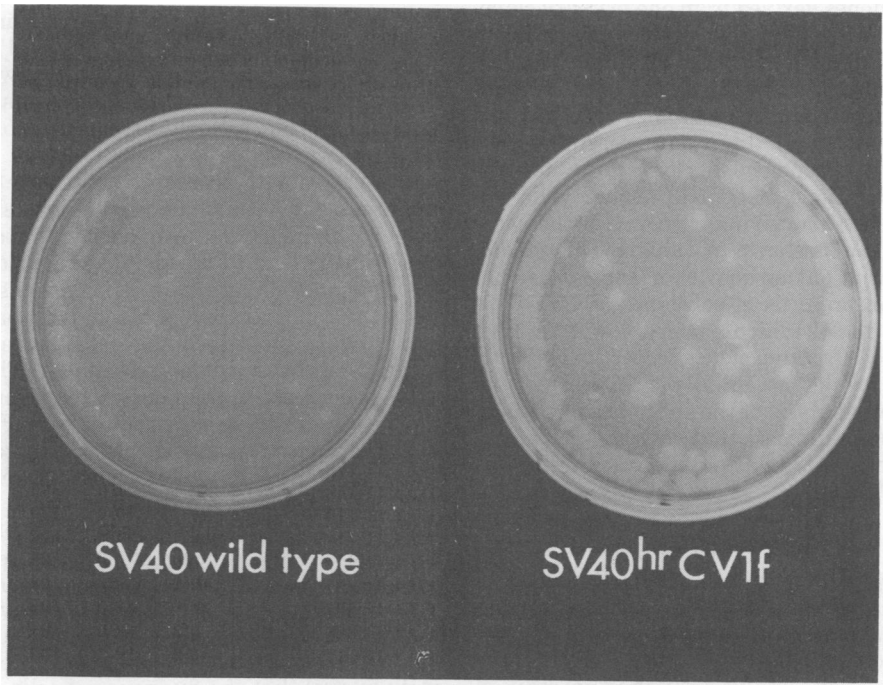

FIG. 4. *Plaque morphology of host range and wild-type virus on CV1f.*

certain fraction of normal cells are always in a physiological state that permits them to escape SV40 infection; (ii) the selection process itself (presumably SV40 infection) induces resistance in a fraction of the cells, (iii) resistant cells arise spontaneously in the permissive cell population as a result of a heritable change. Fluctuation analysis devised by Luria and Delbruck (17) is a classic method for distinguishing the first two kinds of alternatives from the third. If resistant cells arise spontaneously by a heritable change, the number of surviving cells produced by different clones of CV1 will show a much greater fluctuation than will multiple samples from any one clone. That fluctuation reflects the time during the growth of a normally susceptible clone of cells at which the first resistant cell arises. A clone in which a resistant cell arose early in its growth will yield a larger number of survivors than will a clone in which the first resistant cell arose late. If, on the other hand, resistance results from a particular physiological state or is induced by the selection process, every cell will have the same probability of becoming resistant. In that case, the growth history of the clones will make no difference; the fluctuation among different clones will be comparable to that among multiple samples from any one clone.

To distinguish among these possible mechanisms, fluctuation analysis was applied to a group of clones derived from one primary clone, CV1-13. Analysis of the number of surviving clones indicates a considerably larger fluctuation (as indicated by the variance) for different clones than for multiple samples from any one clone (Table 3). If these data represent true fluctuation, secondary subclones from any one primary subclone of CV1-13 should yield the same range of surviving clones with the same overall mean as shown in Table 3. This prediction is borne out as shown in Tables 4 and 5; fluctuation analysis of subclones of CV1-13-30 and CV1-13-35, which yielded low and high numbers of surviving clones respectively (Table 3), give results essentially identical to the fluctuation analysis of the subclones of CV1-13. These results indicate that resistant cells arise spontaneously in the population as a result of a heritable change.

TABLE 3. *Fluctuation analysis of CV1-13 subclones*

Subclone	Survivors[a]	Variance	F test[b]
CV1-13-22	118	130	24
CV1-13-23	5.4	3.2	984
CV1-13-25	15.4	30	104
CV1-13-30	1.2	.6	5,248
CV1-13-31	103	50	63
CV1-13-34	143	328	9.6
CV1-13-35	112	87	36
CV1-13-36	82	156	20
CV1-13-37	14.6	2.3	157
CV1-13-38	9.2	7.3	431
Mean	60.3		
Variance	3,149		

[a] Confluent monolayers of CV1-13 subclones were infected and 24 h later were replated to 5.0×10^5 cells/90-mm plate. The multiplicity of infection was 20 ± 1.8 SV40 PFU per cell. Surviving clones were stained with hematoxylin 21 days after infection. Values are the average of five plates.

[b] F test is the ratio of variance for all cloned lines (3149) to that for individual cloned lines. For 9×4 degrees of freedom the values of F that one would expect to exceed by chance alone 1% and 5% of the time are 14.7 and 6.0, respectively (15).

DISCUSSION

Our objective in this line of research is to investigate SV40 and host interactions using the general approach of isolating and characterizing host cells that no longer permit SV40 to grow. Detailed analysis of host-introduced blocks should help to elucidate the mechanism of SV40 infection in much the same way as does the complementary analysis of infections by mutant virus. Given appropriate kinds of selective or screening techniques, it seems reasonable (based on results from an analogous approach in bacteria-bacteriophage systems) that several different classes of SV40-resistant cells might be isolated. The results reported in this paper characterize the most straightforward selection method for SV40-resistant cells; isolation of cells that have survived an exposure to SV40 virus.

The experimental parameters that influence the frequency with which permissive cells survive SV40 infection have been defined. Our experiments lead to the simple conclusion that the frequency of surviving cells depends solely upon the concentration of SV40, both parental and progeny, to which the cells are exposed during the course of the experiment. The continuous decrease in frequency of survivors with increasing SV40 concentration (see Fig. 1), which at first may seem surprising, is expected since surviving cells are only partially resistant to SV40. Because this partial resistance results from a uniformly reduced probability of infection, increasing numbers of resistant cells are infected productively (and killed) at increasing concentrations of SV40. A previous investigation of these parameters by Hahn and Sauer (14) led to a relatively more complex interpretation, including (i) that the frequency of cells surviving infection depends on the occurence of sev-

TABLE 4. *Fluctuation analysis of CV1-13-30 subclones*

Subclones	Survivors[a]	Variance	F test[b]
CV1-13-30-2	1.0	.5	28,900
CV1-13-30-3	11.6	6.8	2,130
CV1-13-30-5	354	417	35
CV1-13-30-6	5.6	7.3	1,982
CV1-13-30-14	107	23.2	624
CV1-13-30-18	7.0	7.5	1,929
CV1-13-30-19	41	65.7	220
CV1-13-30-22	21	44.5	325
Mean	68.5		
Variance	14,468		

[a] Confluent monolayers of CV1-13-30 subclones were infected and 24 h later were replated to 5.0×10^5 cells/90-mm plate. The multiplicity of infection was 25 ± 1.6 SV40 PFU per cell. Surviving clones were stained with hematoxylin 21 days after infection. Values are the average of five plates.

[b] F test is the ratio of variance for all cloned lines (14,468) to that for individual cloned lines. For 7 × 4 degrees of freedom the values of F that one would expect to exceed by chance alone 1% and 5% of the time are 15.0 and 6.1, respectively (15).

TABLE 5. *Fluctuation analysis of CV1-13-35 subclones*

Subclone	Survivors[a]	Variance	F test[b]
CV1-13-35-1	158	153	19
CV1-13-35-2	75	283	10.5
CV1-13-35-5	18.2	5.7	520
CV1-13-35-6	21.8	10.7	277
CV1-13-35-10	53	16.5	180
CV1-13-35-11	6	.5	5,930
CV1-13-35-12	8.2	19.7	151
Mean	48.7		
Variance	2,966		

[a] Confluent monolayers of CV1-13-35 subclones were infected and 24 h later were replated to 5.0×10^5 cells/90-mm plate. The multiplicity of infection was 15 ± 2.8 SV40 PFU per cell. Surviving clones were stained with hematoxylin 21 days after infection. Values are the average of five plates.

[b] F test is the ratio of variance for all cloned lines (2,966) to that for individual cloned lines. For 6 × 4 degrees of freedom the values of F that one would expect to exceed by chance alone 1% and 5% of the time are 15.2 and 6.2, respectively (15).

eral cell divisions after infection and (ii) that significantly higher frequencies of resistant colonies are obtained when infected cultures are released from contact inhibition 10 to 14 h after infection. We believe the first conclusion is in error because of their failure to appreciate the partial resistance of surviving cells and the role of progeny virus in their selection. The second conclusion is based on experimental results that we have not been able to reproduce (compare our Fig. 2 with their Fig. 3). The basis for this discrepancy is unclear.

SV40-resistant permissive cells are present in the predominantly susceptible cell population before addition of SV40. Fluctuation analysis of a subclone of CV1 cells indicates that resistant cells arise spontaneously during the growth of normal permissive cells. Thus, resistance apparently is not induced by SV40 infection as has been suggested (14). Indeed one previously characterized SV40-resistant permissive line, which also blocks SV40 infection after adsorption and before full uncoating, was isolated by chance from a CV1 cell population without exposure to SV40 (22). Fluctuation analysis does not define the mechanism by which the heritable change to resistance occurs. It could occur (i) by de novo mutation; (ii) by change in the chromosome complement that affects gene dosage or permits expression of a recessive mutation that is normally present in all CV1 cells; or (iii) by a regulatory change that turns on or off some critical host function. There is some experimental support for resistance resulting from a change in the chromosome complement. Robb and Huebner, in the study cited above (22), correlated the resistant state of their cell line with a decreased chromosome number (about 60 versus the normal CV1 complement of 108). In addition, CV1e and CV1n, the two resistant lines reported in this paper that have been karyotyped, also have a decreased complement of about 90 chromosomes per cell (E. Eicchorn and J. H. Wilson, unpublished data).

Resistant cells that are selected as survivors of an SV40 infection constitute a single major class that blocks SV40 infection at an early stage. All the surviving clones we have characterized are only partially resistant to SV40 infection. Most of the surviving clones isolated by our selection method are less than 10% as susceptible as the parent cells to SV40 infection; 5 to 10% are less than 1% as susceptible. Cells that are absolutely resistant to SV40 infection do not occur spontaneously in our CV1 line at a frequency we can detect. Sixteen independently arising resistant clones have been characterized for the block they present to SV40 infection. All 16 lines are resistant to virus but are fully susceptible to viral DNA infection. The block does not appear to be at the stage of SV40 attachment, since these resistant lines adsorb SV40 normally. Moreover, in the resistant lines that have been tested, the eclipse of SV40 occurs just as in wild-type CV1 cells. Though the stage of infection with which eclipse is associated is uncharacterized for SV40, it occurs after

adsorption. Thus, the block to SV40 infection in these resistant lines occurs at an early stage after adsorption and eclipse but before full uncoating. (Note that this interpretation involves the assumption that naked SV40 DNA, which is applied to cells in the form of a DNA:DEAE-dextran complex, joins the normal pathway of SV40 infection at the stage of full viral uncoating. This assumption, though reasonable and often made, is untested.)

Host range viruses that grow more efficiently on resistant cells than wild-type SV40 have been isolated and partially characterized. Their increased efficiency of plaque formation and characteristic large plaques indicate that they carry a mutation that partially compensates for the defect in the resistant cells. This compensating mutation apparently affects a structural component of the virus, since host range and wild-type DNAs are equally infective. Preliminary experiments indicate that the host range mutation maps in the gene for the major capsid protein of SV40 (W. S. Pollard and J. H. Wilson, unpublished data). Because these host range viruses plaque with equal efficiency on three different resistant lines it is likely that the block to infection in these three lines and perhaps all the resistant lines is the same. Additional studies on the host range viruses and the resistant cell lines to determine the site of the defect in the resistant cells are in progress.

Only one other SV40-resistant permissive clone that was selected by exposure to SV40 has been characterized (21). Although that clone was isolated from a mixed culture of surviving cells that had been carried for several passages in the presence of SV40, it, like the resistant clones reported here, was partially resistant to virus but fully sensitive to SV40 DNA. Thus, cells that survive an SV40 infection, whether isolated immediately or after long-term exposure to SV40, appear to fall into one major class. However, studies on SV40-transformed permissive cells suggest there should be a minimum of two classes of resistant cells: those that are resistant only to virions (the major class), and those that also are resistant to infectious DNA (1, 8, 16, 25). Most SV40-transformed permissive lines are resistant to SV40 virion infection but are sensitive to infection by SV40 DNA, indicating that they block SV40 infection at an early stage. However, two transformed lines are also resistant to infection by SV40 DNA, suggesting that they block infection at a step subsequent to uncoating (1, 25). Most transformed permissive lines have been isolated after long-term culturing in the presence of SV40. In the study cited above (21), three of eight cultures like the one from which the resistant clone was isolated became completely transformed by passage 30. The absence of the second class of resistant cells from among the lines reported here suggests either that it occurs at a low frequency or that it is peculiar to the transformation procedure or the transformed state. What role, if any, the presumptive integrated SV40 genome plays in the resistant state of transformed permissive cells is unknown.

ACKNOWLEDGMENTS

We wish to thank Janell Jenkins, June Hoshi, and Loise Saunders for their expert technical assistance. We also acknowledge and appreciate the critical reading of the manuscript by D. Roufa and J. Butel.

This work was supported in part by research grants from the U. S. Public Health Service (GM-13235, CA-15743, GM-00081), the American Cancer Society (VC-23C, IN-270), and the Damon Runyon-Walter Winchell Cancer Fund (DRG-1264). A portion of this work was completed while J. H. W. was a Fellow of the Damon Runyon-Walter Winchell Cancer Fund and M. D. was a Fellow of the National Science Foundation and of the National Institutes of Health. J. H. W. is a Research Career Development Awardee (CA-00058).

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