

The Stable Classes of Transformed Cells Induced by SV40 Infection of Established 3T3 Cells and Primary Rat Embryonic Cells

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When SV40 is injected into neonatal or weanling hamsters, it induces a variety of malignant tumors (Eddy et al. 1962; Eddy 1964; Diamondopoulos 1973). The initial event in this process is thought to be the transformation of a single cell by a single virus particle. This transformation event can be observed *in vitro* by use of a number of selective assays (Todaro et al. 1964; Macpherson and Montagnier 1964; Stoker 1968; Smith et al. 1971) in cells from several species of mammals (Shein and Enders 1962; Rabson and Kirschstein 1962; Black and Rowe 1963; Diderholm et al. 1966).

Previous studies have suggested that several distinct patterns of transformed behavior may result from *in vitro* infection by SV40 (Black 1966; Smith et al. 1971). We have systematically investigated the transformation of two kinds of mammalian cells by SV40. In doing this we have applied several assays of *in vitro* growth control to randomly picked clones of cells arising after SV40 infection. In this study both highly susceptible heteroploid 3T3 mouse fibroblasts and diploid cultures of embryonic rat cells have been used. The latter cells have the advantage of allowing one to monitor establishment into cell lines as well as morphological transformation (Hayflick and Moorhead 1961).

The data presented in this paper demonstrate that clear differences are seen in the classes of transformed cells induced by infection of the established or primary cultures. Furthermore, both cell cultures give rise to transformants which express only some of the changes of *in vitro* growth properties commonly associated with SV40 transformation.

MATERIALS AND METHODS

Virus. A plaque-purified sample of SV40, strain 776, titering 5×10^8 PFU/ml on BSC-1 cells, was used in all transformation studies. On 3T3 cells this sample had a focus-forming titer of 7.5×10^4 FFU/ml. Infections were carried out by rinsing cell cultures with PBS (phosphate-buffered saline), adding 0.2 ml virus lysate per 60-mm petri dish for 2 hr at 37°C, rinsing with PBS, and then adding DME (Dulbecco's modified Eagle's medium) containing 10% serum.

Mouse cell cultures. Methods used in cloning and

culturing mouse cell lines have previously been described (Risser and Pollack 1974). The culture medium was 90% DME (Gibco H21), 10% calf serum (Colorado Serum Co. or Microbiological Assoc.) containing 50 µg/ml Gentamicin (Schering) or 100 units/ml penicillin and streptomycin (Gibco).

Primary rat embryonic cultures. Embryos were surgically removed from 14- or 15-day pregnant rats of the Fischer inbred strain (CDF albino from Charles River Supply Co.) and minced with fine scissors. After rinsing in PBS, tissue was trypsinized for 10-15 min at 37°C in PBS containing 0.25% trypsin (5-10 ml of trypsin solution/embryo). Suspended cells were decanted and trypsin neutralized by the addition of 5% fetal bovine serum (Rehiss-Rehatuin). Cells were pelleted by centrifugation at 200g for 10 min and rinsed in PBS. Cells were suspended in 90% DME, 10% fetal bovine serum containing 100 units/ml penicillin-streptomycin or 50 µg/ml Gentamicin and plated at appropriate densities. Approximately 5-10% of the cells in the final cell suspension adhered to the petri dish (Risser and Pollack, unpubl.).

Transformation assays. All assays were carried out as described (Risser and Pollack 1974) except that fetal bovine serum was substituted for calf serum when rat cells were used.

Virus rescue by cell fusion. The method of Koprowski et al. (1967) was applied using BSC-1 cells as the permissive cell. After cell fusion, cells were plated on petri dishes for lysates or on monolayers of BSC-1 or CV-1 cells for infectious center assays. The next day infectious center plates were overlaid with medium containing 2% fetal bovine serum, 98% DME, antibiotics and 0.9% agar (Difco-Bacto). Plates were stained at 10 days with the same medium containing 0.01% neutral red and scored at 14 days for plaques. Fused cells were incubated for 1 week at 37°C, frozen, and lysates prepared by sonication, centrifugation and filtration through 0.22-µ filters (Swinnex-Millipore Co.). Virus titers were measured as previously described (Risser and Pollack 1974).

Chromosome analysis. Chromosomes were prepared and counted as described by Pollack et al. (1970).

Fibrinolysin assays. A modification (Pollack et al., unpubl.) of the procedure of Unkeless et al. (1973) was used to assay the production of total (cell-bound plus secreted) plasminogen activator by living cells and the presence of this activator in detergent-treated lysates of cell cultures.

RESULTS

Classes of SV40-transformed 3T3 cells. To assay the effects of SV40 on 3T3 cells without selecting for a particular class of transformed cells, we carried out the following experiment. 3T3 cells were infected with a high multiplicity of SV40 and plated sparsely to allow individual infected cells to form colonies. Forty clones were picked without regard to morphology from infected plates, four clones were picked from mock-infected plates and four dense foci were picked from infected plates to serve as normal and transformed controls. All clones were tested for viral T antigen, saturation density and doubling time in 10 and 1% calf serum, colony formation on monolayers of 3T3 cells, and colony formation in methyl cellulose suspension.

Three patterns of viral T antigen staining were observed: no intranuclear fluorescence, weak intranuclear fluorescence with considerable heterogeneity from cell to cell, and intense intranuclear fluorescence (Fig. 1). These staining patterns can be used to classify clones as T antigen negative, T antigen intermediate and T antigen positive. Complement fixation assays performed by Dr. M. Osborn have confirmed the immunofluorescence assays. The proportion of intensely staining cells in negative or intermediate lines is not altered by treatment with iododeoxyuridine (IdU) or bromodeoxyuridine (BrdU), culture crowding, addition of cortisol or dexamethasone to the culture medium or pulses of ultraviolet light.

When the growth properties of these clones were compared, a surprising result was found. In any particular assay, control lines were well separated, the distinction between normal and transformed

behavior being quite clear. This was not the case with experimental lines. A range of saturation densities and plating efficiencies in methyl cellulose suspension or on normal monolayers was seen (Fig. 2). Furthermore, most experimental lines grew rapidly in 1% calf serum whereas the four mock control lines and parental 3T3 cells did not. Several of the experimental lines, which showed this altered serum requirement, lacked viral T antigen. Other growth properties correlated quite well with T antigen staining, as shown in Figure 2. From these data four distinct classes of SV40 cells could be distinguished (Table 1), one of which corresponded to standard SV40-transformed cells previously described (Todaro et al. 1964), and one to cells unchanged in any growth assay from 3T3. The two other classes of cells, minimal transformants and intermediate transformants, are in some ways similar to the SV40 lines described by Smith et al. (1971) and Scher and Nelson-Rees (1971). Several lines have been subcloned and passaged for as long as one year in vitro. These lines show the same growth properties as they did on initial testing (Risser and Pollack 1974).

Virus rescue and supertransformation of SV40 lines. The state of the virus in various transformed lines was investigated by the cell fusion technique (Table 2). The three standard transformed lines all yielded SV40 on fusion with permissive monkey cells in amounts comparable to those reported by others (Watkins and Dulbecco 1967; Koprowski et al. 1967; Vogel et al. 1973). The three minimal transformants, however, did not yield infectious virus. Of the three intermediate lines tested, only one

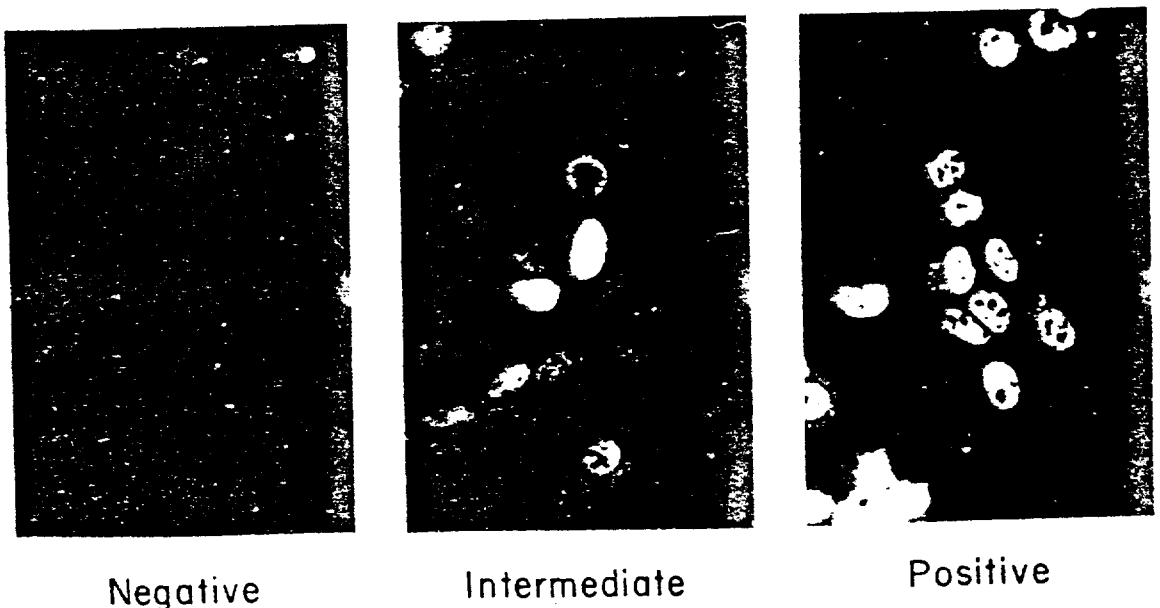


Figure 1. T antigen staining pattern of SV40 3T3 cells. Cells are fixed and stained as described in Risser and Pollack (1974). From left to right the cells are 3T3, SVR clone 16, and SV101.

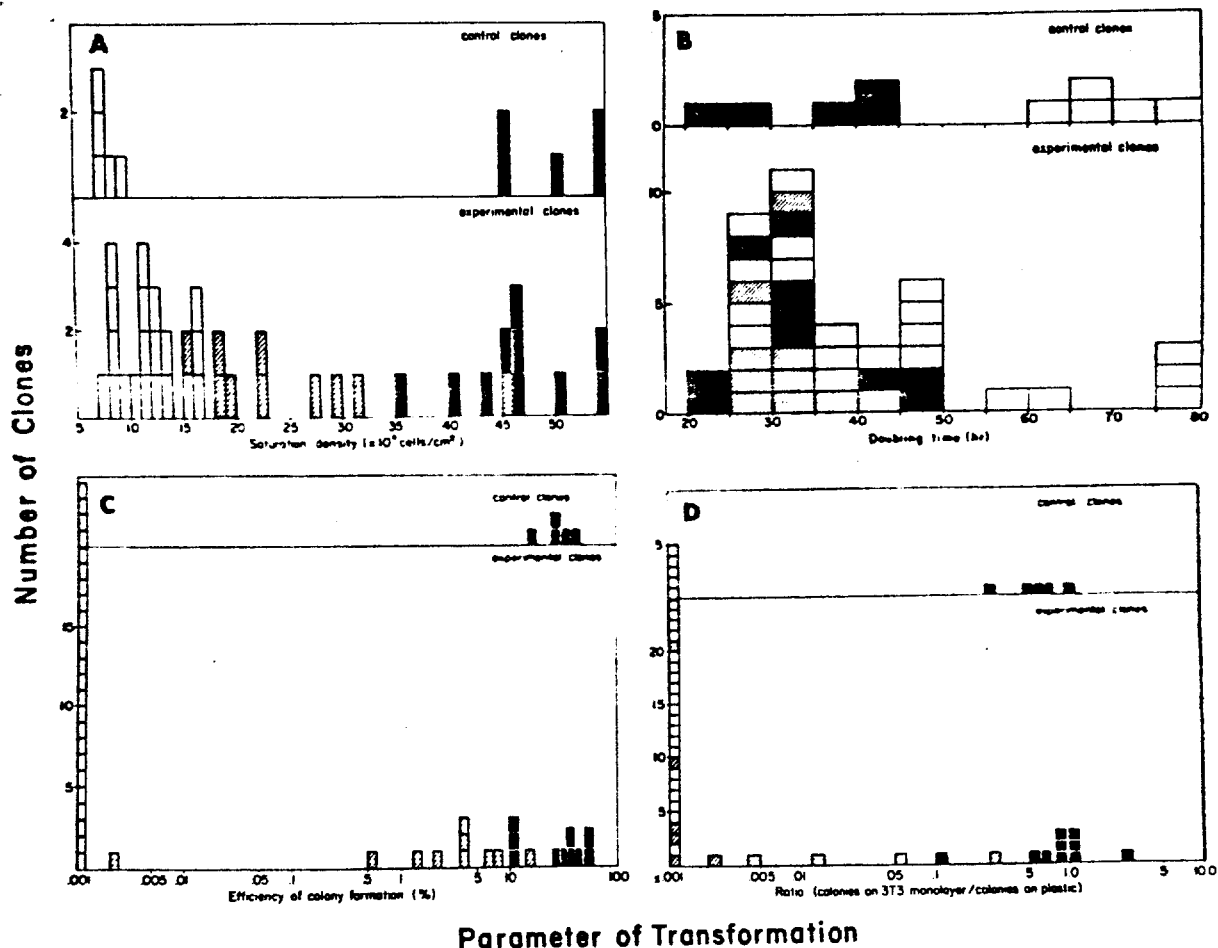


Figure 2. Histograms of growth properties of SV40 3T3 cells. (A) Saturation density in 10% calf serum; (B) doubling time in 1% calf serum; (C) efficiency of colony formation in methyl cellulose suspension; and (D) ratio of the plating efficiency on 3T3 monolayers to the plating efficiency on plastic dishes for each of 40 experimental lines, five 3T3 control lines, and five SV40-infected 3T3 focal lines. Data from Risser and Pollack (1974).

yielded infectious virus; furthermore, the fraction of cells producing virus was approximately 100-fold lower in this line as compared to standard transformed lines. A similar reduction in virus yield

was observed when SV40 was recovered from phenotypic revertants of transformed cells (Vogel and Pollack, unpubl.).

The susceptibility of minimal and intermediate

Table 1. Classes of SV40 Mouse 3T3 Clones

Class	% Clones	T antigen	Saturation density in 10% CS ($\times 10^4$ cells/cm ²)	Doubling time in 1% CS (hr)	EOP in methyl cellulose (%)	Colony formation on normal cells*
Normal	12.5	-	8.5 (7.6-9.5)	78 (55-100)	$\leq .001$	$\leq .001$
Transformed	25	+	47 (35-60)	34 (24-45)	32 (11-58)	.94 (.1-2.6)
Minimal transformed	37.5	-	15 (9.5-16.5)	34 (26-49)	$\leq .001$	$\leq .001$
Intermediate transformed	25	\pm	25 (15-45)	32 (29-41)	4 (.5-14.6)	.03 (.001-.20)
Control						
Normal		-	7.8 (7-9)	70 (60-86)	$\leq .001$	$\leq .05$
Transformed		+	53 (47-60)	37 (26-43)	25 (16-30)	.7 (.25-1.1)

The mean numerical value for each parameter of transformation is reported for each class of transformant, and in parentheses the range of values seen among different clones in each class is given. Data from Risser and Pollack (1974).

* Expressed as the ratio of EOP on 3T3 monolayers to EOP on plastic dishes.

Table 2. Virological Properties of SV40 3T3 Clones

Class	Superinfection		Virus rescue ^a		
	T antigen (%)	focus formation (%)	heterokaryon formation (%)	infectious center	virus titer (PFU/ml)
3T3	75	2.2	N.T.	0	0
Minimal					
SV cl 42	63	2.4	5	0	0
57	72	2.3	5	0	0
95	73	1.1	5	0	0
Intermediate					
SV cl 13 mock-	35	N.T.	4	0	0
infected	35	N.T.			
32 mock-	22	.14 ^b	N.T.	0	0
infected	19	.10 ^b			
63 mock-	26	≤.001 ^b	5	2	2 × 10 ² , 1 × 10 ³
infected	35	≤.001 ^b			
Standard					
SV cl 86			N.T.	N.T.	2.5 × 10 ⁴
114			5	400	1.8 × 10 ⁵
SV101			N.T.	N.T.	2 × 10 ⁴

^a Virus rescue was performed as in Results. After fusion, cells were plated on BSC-1 cells for infectious center assay (reported as infectious centers per 10⁵ cells) or plated in plastic dishes and incubated one week for production of the virus lysate.

^b Cells were infected or mock-infected and plated in medium containing methyl cellulose (Stoker 1968; Risser and Pollack 1974). Colonies were scored at 3 weeks.

transformants to retransformation by SV40 was investigated. Since minimal transformants grow to approximately monolayer density, these were tested in the standard dense focus assay. Intermediate transformants do not maintain a monolayer of cells and hence had to be tested by the suspension culture assay (Stoker 1968). As shown in Table 2, minimal transformants are as susceptible as 3T3 cells to SV40 induction of dense colonies. Intermediate transformants cannot be transformed by SV40 as assayed by colony formation in methyl cellulose suspension, nor can the fraction of cells showing bright nuclear T antigen fluorescence be changed by infection with virus (Table 2). However, we do not know if these cells allow virus adsorption or penetration.

Infection of primary rat cells by SV40. From the preceding work on SV40 transformation of established 3T3 cells, it seems clear that considerable diversity of transformed behavior can be induced by SV40. Two obvious questions arise from this phenomenon. Can this observation be generalized to other cell systems, and is there a way to readily monitor it? In attempting to answer these questions we have turned to primary rat cells. Since primary cells do not form colonies when seeded sparsely and eventually cease to divide on serial passage, one has the additional cellular property of established and prolonged clonal growth from a single cell as an assay of altered behavior (Puck and Marcus 1955; Zaroff et al. 1961; Hayflick and Moorhead 1961).

We have observed that infection of primary rat embryonic cells by SV40 induces the cells to form

isolated colonies under conditions where the mock-infected cells form many fewer colonies (Fig. 3). Fresh primary or secondary cultures were infected with SV40 and plated as in a standard focus assay. The plating efficiency of uninfected cells ranged from 0.1–0.9%; the infected culture plated with an efficiency of 2.5–10%. The variation observed was largely due to different protocols of preparation of primary cultures. In order to obtain a low plating efficiency for uninfected primary cultures, it was important to always maintain the cells below a density of 2 × 10⁴ cells/cm². When cells were initially seeded at higher densities or allowed to grow for several days, plating efficiencies of up to 10% were observed when the cells were replated sparsely.

Among infected cultures, at least two colony morphologies could be distinguished: dense and flat (Fig. 3). Dense colonies resembled standard SV40 foci and consisted of multiple cell layers, whereas most flat colonies consisted of a single cell layer. Among flat colonies, two types could be distinguished: those which consisted of large stellate cells with many multinucleated cells and those which consisted of smaller epithelial or fibroblastic cells with frequent mitoses throughout the colony. The rare colony seen in uninfected plates usually consisted of large stellate cells.

The induction of colony formation by primary cells following SV40 infection is an inefficient event and, surprisingly, not strikingly dependent on virus multiplicity (Table 3). Though dense colony or focus formation is linearly dependent on virus dose, quite low doses of virus were sufficient to stimulate cells to form colonies. Apparently a threshold of about 5% of the total cells can be stimulated to

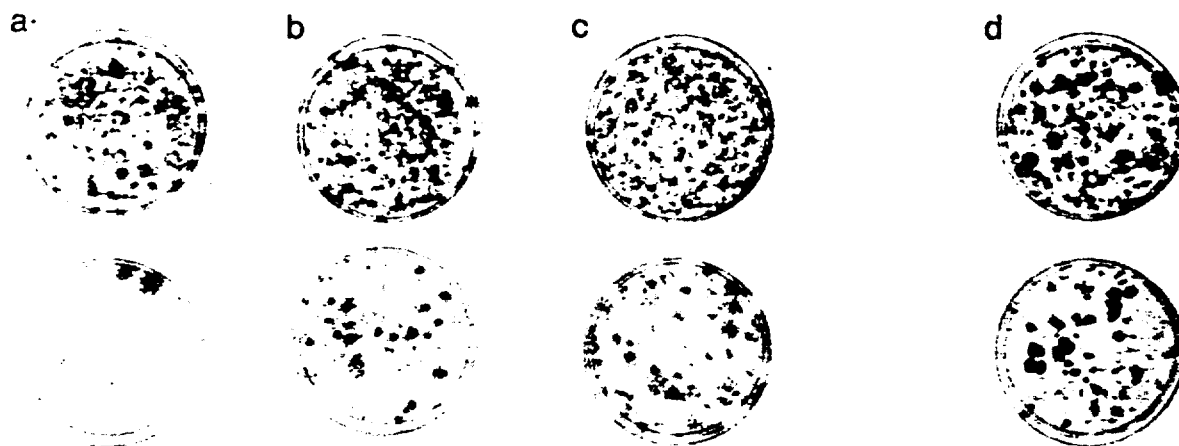


Figure 3. Example of colony formation by embryonic rat cells (group on left). Primary cultures of rat embryonic cells (15th day of pregnancy) were seeded at a density of $\sim 2 \times 10^5$ cells/cm². Approximately 10% of the cells adhered to the petri dish. The following day, cells were rinsed with PBS several times and infected with (a) 0.006 ml of DME (mock) or 0.006 ml SV40 (b, c) virus per cm² of surface area. After 2 hr adsorption at 37°C, virus was removed and 10% fetal bovine serum replaced onto the petri dish. The next day, cells were trypsinized and plated at 10^5 , 10^4 (top row) and 10^3 (bottom row) cells/dish (25 cm²). Medium was changed every 3 days and plates were stained at the end of 2 weeks. A parallel experiment using 3T3 cells is included for comparison (d).

form large visible colonies. At low viral dilutions, some of these colonies are probably the result of proliferation of non-T antigen-forming cells (Table 4).

Establishment of primary rat cells by SV40. We have further investigated the ability of colonies induced by SV40 to synthesize viral T antigen and grow continuously on serial subcultivation. Secondary cells were infected or mock-infected, seeded sparsely on coverslips, and allowed to form isolated colonies over a 2-week period. These colonies were then stained for the presence of viral T antigen. As can be seen from Table 4, a number of these colonies did not contain viral T antigen.

Several colonies from mock-infected or virus-infected plates were cloned from plates using steel cloning cylinders and subcultured in 35-mm dishes containing coverslips. At the end of 2 weeks each clone was scored as to whether it had significantly increased in cell number, and coverslips from the

same petri dish were stained for viral T antigen (Table 4). None of the ten mock-infected clones had grown significantly during this period nor did they contain viral T antigen. None of the infected clones which lacked viral T antigen proliferated, and three subclones which did not proliferate contained a minority of T antigen-positive cells. All clones which proliferated well showed intense intranuclear T antigen fluorescence in greater than 95% of the cells of that clone. Thus in contrast to the observation of minimal transformants with 3T3 cells, we were unable to obtain an established clone of T antigen-negative minimally transformed rat cells from infected primary cultures.

Properties of SV40-transformed rat clones. Twelve clones were selected and characterized for their growth in 10 and 1% fetal bovine serum, their karyotype, their growth in methyl cellulose suspension and their ability to activate plasminogen. The latter property was assayed by the release of

Table 3. Colony Formation and Transformation of Rat Embryonic Cells by SV40

Protocol	MOI (PFU/cell)	T antigen production (%) ^a	Focus formation (%) ^b	Efficiency of plating (%) ^c
3T3 cells	0	0	0	38
	400	95	3.10	37
Rat secondary cells	0	0	0	0.6
	13	0.3	0.01	4.8
	133	3.2	0.09	4.8
	400	11.2	0.17	2.7

^a T antigen-forming cells 2 days after infection.

^b Foci were scored as the number of dense colonies per cells plated on plates receiving 10^4 or 10^3 cells.

^c Efficiency of plating was scored as the number of colonies formed on plates receiving 10^3 cells.

Table 4. T Antigen Production and Cellular Establishment of SV40 Rat Clones

Protocol	Viral T antigen		
	positive	mixed	negative
Infected mock SV40	39	14	7 25
Infected, subcultured mock, nongrowing			10
infected, growing	21	2	0
nongrowing	0	1	10

soluble fibrinopeptides from fibrin-coated dishes in the presence of plasminogen (Unkeless et al. 1973). This activity of fibrinolysis has been correlated with hyperplasia in tissue repair (Astrup 1968) and malignant tumor formation (Reich 1974), as well as the in vitro properties of cellular morphology (Ossowski et al. 1973a) and growth in agar suspension (Ossowski et al. 1973b).

All of these SV40-transformed rat clones were uniformly T antigen positive. We have found that all clones grow rapidly in 10 and 1% serum; furthermore in 10% serum, several SV40 clones continue growing until they detach from the petri dish (Table 5). Secondary uninfected cells do not divide when seeded at the same initial density in 1% serum and grow to moderate saturation density ($10\text{--}15 \times 10^4$ cells/cm²) when seeded in 10% serum. In growth assays in 10 and 1% serum, all SV40 rat lines behave like standard transformants described earlier. All SV40 clones except clone 5 contain a heteroploid karyotype consisting of approximately

70–80 chromosomes, whereas clone 5 shows a pseudodiploid complement of 40 chromosomes (Table 5) compared to 42 found in diploid rat secondary cells.

The twelve clones tested showed a wide range of plating efficiencies in methyl cellulose suspension (<0.001–2%) and considerable variation from clone to clone in the production of fibrinolytic activity (Pollack et al., unpubl.). Comparable levels of fibrinolytic activity were found when living cells were assayed or when detergent-treated lysates were assayed for the presence of plasminogen activator. Colony formation in methyl cellulose suspension was related to the amount of plasminogen activator produced. Thus these two parameters of in vitro transformed behavior appeared well correlated (Fig. 4). All these twelve clones have been in culture approximately 9 months and 11 of the 12 have shown no decrease in their growth rates or changes in fibrinolytic activity or plating ability in methyl cellulose suspension (Pollack et al., unpubl.).

DISCUSSION

The data presented here demonstrate that SV40 can induce several stable patterns of growth control in two mammalian cell systems. Though differences were observed between the response of established or secondary embryonic cells to SV40 infection, the central conclusion of a diverse expression of transformed growth properties seems well founded in both systems.

Perhaps the most pervasive cellular change observed after SV40 infection is in the serum require-

Table 5. Growth Properties of SV40-transformed Rat Clones

Clone	Growth in 1% fetal bovine serum		Mean chromosome number ^b	Efficiency of plating in methyl cellulose (%)	Mean fibrinolytic activity ^c
	saturation density ^a	doubling time (hr)			
Rat embryo cells	N.P. ^d	N.P. ^d	40.7 ± 2	0.001	1.00 (4)
SV cl 1	6	25	N.D.	0.2	7.19 (1)
2	13	33	N.D.	N.D. ^e	N.D. ^e
3	12	33	71.4 ± 10	0.06	1.53 (3)
4	11	35	N.D.	0.80	2.08 (1)
5	14	27	39.8 ± 1	2.60	7.67 (3)
6	9	50	74.4 ± 11	0.01	2.17 (1)
7	10	28	82.8 ± 10	N.D. ^e	N.D. ^e
8	20	30	76.7 ± 4	2.15	5.14 (1)
9	14	27	77.7 ± 5	1.26	12.31 (4)
10	14	30	74.5 ± 19	0.30	2.19 (1)
11	14	38	76.7 ± 5	0.002	0.64 (1)
12	11	33	85.9 ± 11	0.010	1.44 (4)

^a Saturation density = cells/cm² × 10⁴.

^b Chromosome counts performed on 20 metaphase spreads. The mean chromosome number ± standard deviation is given.

^c Fibrinolytic activity is reported as activity relative to rat embryonic cells. Activity is calculated as cpm I¹²⁵ released/10⁵ cells/10³ available I¹²⁵ cpm/hr. The range of activity in rat embryonic cells is 36–198 cpm/10⁵ cells/10³ available cpm/hr. Parentheses indicate the number of trials.

^d Not possible. Seeded at 2×10^3 cells/cm² rat embryonic cells do not divide in 1% fetal bovine serum.

^e Not done.

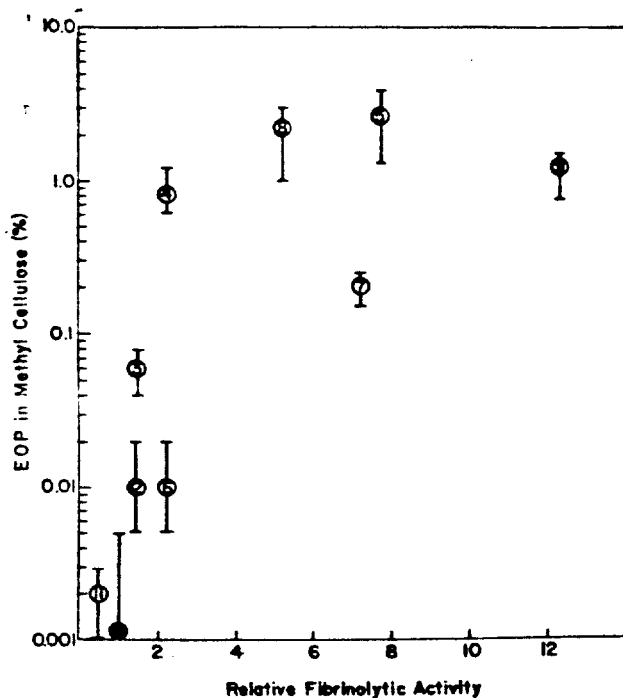


Figure 4. A plot of plating efficiency in methyl cellulose suspension vs. relative fibrinolytic activity. Cells from rat clones were seeded at densities of 10^5 , 10^4 , 10^3 and 10^2 cells/plate in medium containing 1.2% methyl cellulose. Cultures were fed weekly and scored after 3 weeks for the number of colonies of more than 200 cells. Fibrinolytic activity was assayed by the procedure of Unkeless et al. (1973) and corrected to cpm released/ 10^5 cells/ 10^5 available cpm/hr. Fibrinolytic activity is expressed relative to that found in primary embryonic cells.

ment of infected cells. This change, as monitored by growth in 1% serum, is demonstrated by all classes of stable transformed cells. In 3T3 cells, several lines show only this alteration with no other evidence of viral expression, e.g., T antigen, high saturation density. Such a class of cells was not obtained from primary cells, perhaps because T antigen synthesis is necessary for establishment of primary cells into cell lines by SV40.

Probably related to a change in serum requirements is the ability of primary cells to form colonies in sparse culture following SV40 infection (Puck and Marcus 1955; Zaroff et al. 1961). Several morphological classes of colony formers were observed, some of which contained viral T antigen and some of which did not. When such colonies were picked, only those expressing viral T antigen could be serially subcultured, arguing strongly that those changes which T antigen production reflects are necessary for permanent establishment by SV40. The cells of colonies which did not express viral T antigen are possibly analogous to abortive transformants (Stoker 1968; Smith et al. 1971) or are the result of conditioning effects by other colonies growing in the same petri dish. These observations suggest, at the minimum, that changes in serum

requirements of cells can take place in the absence of other changes in growth behavior; and when other changes in growth control do take place, serum requirements are of necessity altered. A similar conclusion has been drawn from studies of revertant lines of SV40-transformed 3T3 cells (Vogel and Pollack 1973).

A second series of parameters of growth control relate viral gene expression, e.g., T antigen, to transformed behavior. In comparing the behavior of 3T3 and rat transformed lines in terms of saturation density, T antigen and growth in methyl cellulose suspension, some significant differences were noted. All SV40 rat lines were uniformly T antigen positive and grew to high or unmeasurable saturation densities in 10% serum. This was not the case with all SV40 3T3 lines. An additional selective pressure was placed on infected primary rat cells in that they were required to grow repeatedly in sparse culture. In order to obtain intermediate or minimal lines from infected primary cells, it would apparently be necessary to culture them under less selective conditions. SV40 rat lines did show significant differences in their colony-forming ability in methyl cellulose suspension, however, and in this manner resembled the spectrum of transformed responses seen with 3T3 cells.

The correlation of both extracellular and intracellular levels of plasminogen activator with the ability of SV40 rat lines to grow in methyl cellulose suspension is probably more than fortuitous, as neither property was knowingly selected for. Ossowski et al. (1973a,b) have observed that inhibition of fibrinolytic activity or removal of plasminogen from the medium reduces the plating efficiency of SV40 hamster cells in agar by about 75%. These two observations suggest that fibrinolytic activity is both necessary and sufficient for the formation of colonies in suspension culture by SV40-transformed rat cells.

The cellular basis for these diverse growth patterns is not at all clear. Preliminary results suggest that synchronized secondary rat cells infected during phases G1 or S show the same pattern of colony induction by SV40 as asynchronous populations (Risser and Pollack, unpubl.). Furthermore, procedures which enrich for fibroblasts and thus yield more homogeneous cultures for infection do not affect the pattern of colony induction by SV40 (Risser and Pollack, unpubl.). It seems likely that some of the diversity seen among SV40 rat lines is due to progressive changes which occur during propagation of the cells from single clones. As was shown in Figure 3, most clones initially induced by SV40 have a flat morphology. If the cells in these clones continue to express T antigen, the resulting lines derived from them most commonly grow to high saturation densities in 10% serum. Similar progressive changes have been observed in vitro in polyoma-infected hamster lines (Vogt and

Dulbecco 1963) and among some SV40 3T3 intermediate lines (Risser and Pollack 1974).

Many rat clones do not, however, acquire the additional property of growth in methyl cellulose suspension, and this remains the most stringent assay for SV40 transformation in both 3T3 and rat primary systems. The plating ability of transformants derived from primary cultures of rat embryo cells in methyl cellulose suspension is correlated with the production of plasminogen activator. The production of this enzyme has also been found closely associated with malignant tumors of various species though not with normal cells or benign tumors. These observations suggest that colony formation in methyl cellulose suspension may select and define a class of SV40 transformants more closely related to malignant cells.

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