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Two Classes of Revertants Isolated from SV40-transformed 3T3 Mouse Cells

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A major unresolved problem is to determine which, if any, of the selective and non-selective differences between 3T3 and SV40-transformed 3T3 cells are the consequence of the continued expression of integrated viral genes (Eckhart, Dulbecco and Burger 1971). Our approach to this problem has been to isolate and characterize sublines that have reverted to resemble 3T3 in some or all of these differences, and to then compare SV40 viral expression in revertants to that in transformants.

Properties of Transformed Cells

Transformants isolated for their ability to grow to high cell densities on top of 3T3 monolayers (Todaro and Green 1963; Todaro, Green and Goldberg 1964) also grow to high saturation densities when cloned and propagated in 10% calf serum on plastic. We term clones with these two properties density transformants.

Normal 3T3 cells grow poorly in 1% calf serum (Holley and Kiernan 1968; Dulbecco 1970), gamma-globulin-free serum (Jainchill and Todaro 1970) or gamma-globulin-free serum depleted by incubation with confluent Balb-3T3 cells (Smith, Scher and Todaro 1971). Following infection with SV40, colonies of cells arise with a diminished serum requirement for growth. These colonies can grow in either 1% calf serum (Risser and Pollack this volume) or agamma-depleted calf serum (Smith et al. 1971). We term clones having this reduced serum requirement for growth serum transformants. Serum transformants may also be density transformed, but a significant fraction maintain a low saturation density in 10% calf serum (Scher and Nelson-Rees 1971; Risser and Pollack this volume).

SV40 infection of 3T3 also yields a small minority of cells with the ability to grow in methylcellulose or soft agar (Black 1966). These colonies can be recovered with a micropipette and cells from these colonies are capable of establishing new colonies when suspended in Methocel.

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SV40-transformed cells contain an intranuclear SV40-specific tumor antigen (Black et al. 1963). Transformed cells have a lower intracellular cyclic AMP concentration than normal cells (Otten, Johnson and Pastan 1971; Sheppard 1972; Seifert and Paul 1972). While neither the presence of viral products nor the changes in cyclic AMP concentration have yet been used to select transformants directly, both are found to have occurred in most transformants derived by the above selective assays.

Selection of Revertants

Variant sublines selected specifically for reversion in one of the transformed properties have been described (Pollack, Green and Todaro 1968; Culp, Grimes and Black 1971; Culp and Black 1972; Wyke 1971; Ozanne and Sambrook 1971; Ozanne 1973; Vogel and Pollack 1973; Vogel, Risser and Pollack 1973). Such revertant lines are isolated by negative selection from populations of transformed cells exhibiting all three transformed growth properties. In this technique culture conditions are such that cells with a single transformed property are killed, but cells resembling 3T3 in that property survive and can be recovered after removal of the toxic agent.

Density revertants grow to low saturation densities in excess amounts of serum (Pollack et al. 1968; Culp et al. 1971; Vogel et al. 1973), anchorage revertants are unable to form colonies in Methocel (Wyke 1971), and serum revertants cannot grow in 1% calf serum (Vogel and Pollack 1973).

We have asked whether reversion in one transformed property necessarily leads to reversion in the other transformed properties. Also we have attempted to characterize the amount and degree of expression of the SV40 genome in the revertant lines in order to ask whether the revertant properties are accompanied by a defect in SV40 expression. All the revertant lines discussed below were derived from a single clone of SV40-transformed 3T3 cells, SV101.

RESULTS

Growth Properties of Serum Revertants

Normal and transformed cells show markedly different responses to 1% calf serum (Table 1). Normal 3T3 cells grow poorly in 1% calf serum with a doubling time of 90 hours, whereas SV101 grows relatively well with a doubling time of 30 hours.

Serum revertants are isolated by plating SV101 in either 1% calf serum or 10% agamma-depleted calf serum (Smith et al. 1971) and adding BrdU to kill dividing cells (Vogel and Pollack 1973). Revertants isolated in 1% calf serum are designated as LsSV, and revertants isolated in 10% agamma-depleted serum are designated as AγSV. Neither type of serum revertant can grow at all in 1% calf serum (Fig. 1).

Although these lines were selected only for serum reversion, they have also reverted to a low saturation density (Table 1). This second reversion is not expected. Its consistent occurrence suggests that a high serum requirement for growth is not compatible with growth to a high cell density.

The two classes of serum revertants differ in their anchorage requirement for growth. Revertants isolated in 1% calf serum (LsSV1 and LsSV2) do not form

Table 1
Growth Properties of Mouse Cell Lines in 10% and 1% Calf Serum

	Line	*Anchorage (growth in Methocel*)*	*Saturation density in 10% calf serum (cells/ cm² × 10⁴)*	*Doubling time in calf serum (hr)*	
				1%	*10%*
Parent lines	3T3	0.001	5	90	21
	SV101	20	>45 (peels)	30	16
Serum revertants	AγSV4	2	15	>120	22
	AγSV5	11	10	>120	21
	LsSV1	0.001	9	>120	22
	LsSV2	0.04	12	>120	22
Density revertants	FlSV101	0.01	9	36	22
	BuSV2	0.02	13	50	25
	BuSV3	0.05	15	45	24

Cells were seeded in 4 ml of Methocel medium containing 10% calf serum and incubated for 3 weeks, with 4 ml of fresh medium added every week. Colonies larger than 0.3 mm in diameter were counted (Vogel et al. 1973).

Cells were plated at 0.1–0.2 × 10^4 cells/cm^2 in medium containing the appropriate serum concentration. Counts were done daily by trypsinizing a plate and counting on a Coulter Counter. The medium was changed every 3 days.

* Visible spherical colonies (greater than 300 micron diameter) per 100 cells cultured in Methocel for 21 days.

colonies in Methocel, while revertant lines isolated in agamma-depleted serum form colonies in Methocel with a high efficiency (Table 1). Thus the Aγ revertants represent a unique class of cells which are transformed in only the anchorage property (Table 2).

Growth Properties of Density Revertants

Density revertants were isolated by plating transformed cells in 10% calf serum and killing the cells capable of growing in dense culture with FdU (Pollack et al. 1968) or BrdU (Vogel and Pollack 1973). FlSV101 is a density revertant isolated with FdU, and BuSV2 and BuSV3 are revertants isolated with BrdU. These lines have reduced saturation densities, comparable to 3T3 (Table 1).

Reversion in saturation density can be accomplished without affecting the transformed serum requirement, since density revertants grow in 1% calf serum with a doubling time similar to that of SV101 (Fig. 1). Density revertants show that the properties of serum transformation and density transformation are closely, but not inextricably, linked, since in these lines density inhibition of growth is compatible with a low serum requirement.

Relation of Saturation Density to Serum Requirement

For all lines examined saturation density is dependent on serum concentration (Fig. 2). The data in Fig. 2 are in agreement with the hypothesis that alterations in

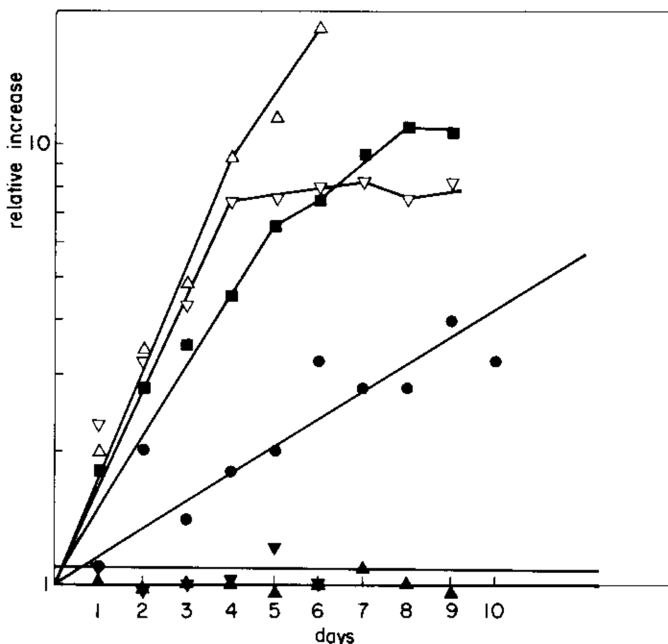


Figure 1
Growth of serum revertants in 1% calf serum. Cells were plated at 0.1–0.2 × 10^4 cells/cm^2 in 35-mm dishes. Cells per plate were determined daily on a Coulter Counter. The medium was changed every 3 days. (•) 3T3; (△) SV101; (▽) FlSV101; (▪) BuSV3; (▲) LsSV2; (▼) AγSV5.

saturation density are the result of changes in serum requirement (Holley and Kiernan 1968). However, while the slopes of the graphs for SV101, 3T3 and the density revertants BuSV3 and FlSV101 are similar (Fig. 2a), the serum revertants LsSV2 and AγSV5 have steeper slopes than all the other lines (Fig. 2b).

This implies that serum revertants utilize serum in a manner different from either the parent lines or the density revertants. Specifically these data distinguish two classes of cells, which have similar saturation densities in 10% calf serum but respond quite differently to 1% calf serum. The serum revertants LsSV2 and AγSV5 fail to grow in 1% calf serum and grow to low saturation density in 10% calf serum, whereas the density revertants FlSV101 and BuSV3 have a low saturation density in 10% calf serum despite their ability to grow well in 1% calf serum.

Normal 3T3 cells cease DNA synthesis and mitosis upon forming a confluent monolayer, but SV101 cells continue cell division after confluence is reached (Fig. 3; Table 3). We have examined the regulation of cell division in 10% calf serum of one density revertant, FlSV101 (Pollack and Vogel 1973). At confluence FlSV101 cultures show a marked decrease in the fraction of mitotic cells (Fig. 3; Table 3). While 15% of FlSV101 cells synthesize DNA at confluence, the rate of DNA synthesis at confluence is only a few percent of the rate of synthesis in logarithmically growing cells (Table 3). We conclude that FlSV101 regulates its saturation density in a manner similar to 3T3.

Table 2
Properties of Revertants Isolated from SV101

Line	*Saturation density*[a]	*Serum requirement*[b]	*Anchorage requirement*[c]
3T3	Normal	Normal	Normal
SV101	Transformed	Transformed	Transformed
Density revertants selected with:			
FdU	Normal	Transformed	Normal
BrdU	Normal	Transformed	Normal
Serum revertants selected in:			
1% calf serum	Normal	Normal	Normal
Aγ-depleted calf serum	Normal	Normal	Transformed

[a] Normal cells have saturation densities less than 15×10^4 cells/cm^2 in 10% calf serum.

[b] Assayed by growth in 1% calf serum. Normal cells have doubling times greater than 90 hr; transformed cells double in 35 hr or less.

[c] Assayed by ability to form a colony in Methocel. Normal cells do not form colonies in Methocel; transformed cells do.

We do not know how the serum revertants maintain a low saturation density. They may show a density-dependent decrease in DNA synthesis at confluence, or they may continue to proliferate and shed at confluence (Scher and Nelson-Rees 1971).

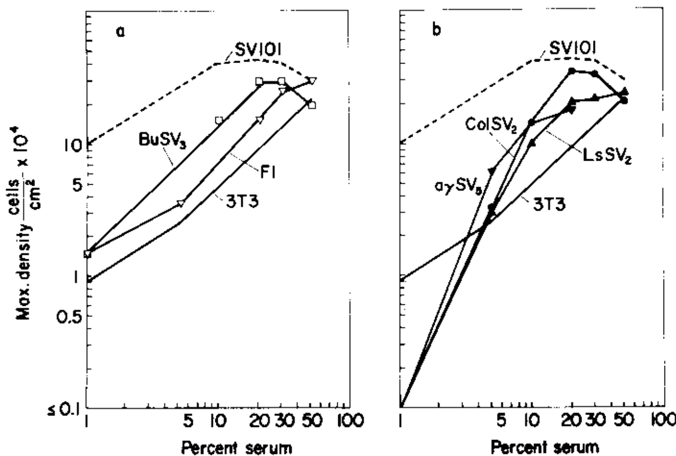


Figure 2
Saturation density as a function of serum concentration. Cell growth was determined as described above in varying concentrations of serum. ColSV2 is a revertant isolated with colchicine (Vogel et al. 1973). From Vogel and Pollack (1973) with permission of *J. Cell. Physiol.*

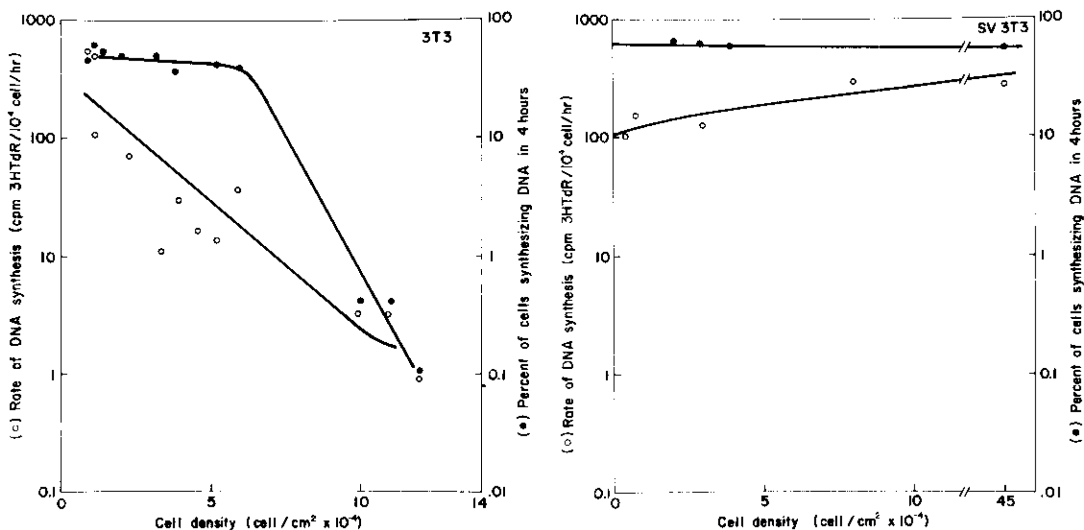
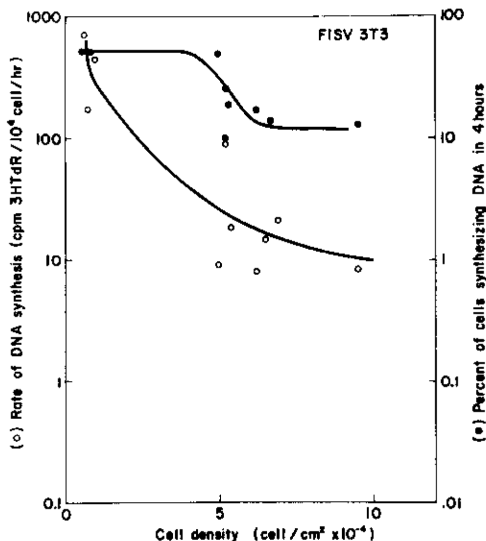


Figure 3

Fraction of cells synthesizing DNA and rate of thymidine incorporation at different cell densities. Cells were inoculated into 9.6-cm^2 dishes containing sterile 12-mm circular glass coverslips (area = 1.13 cm^2). After 4 hr exposure to 2.5 μCi/ml [^{3}H]thymidine in the presence of 10^{-5} M cold thymidine, coverslips were washed twice in PBS, fixed in ethanol-acetic acid (2:1), air-dried, and mounted cell-side up on microscope slides. The slides were dipped in melted (40°C) Kodak NTB-2 emulsion, air-dried for 2 hr, stored at 4°C for 48 hr, developed in Dektol, and permitted to dry and harden overnight. The developed slides were then stained in hematoxylin (Harris), blued in $LiCl_2$, cleared through alcohol to xylene, mounted in Permount, and examined at 500×, phase contrast (Zeiss). Silver grains were seen only overlying nuclei, which had been stained blue by the hematoxylin. Cytoplasmic localization of [^{3}H]thymidine, a sign of contamination by mycoplasma, was never observed (see Fig. 2).



After exposure to 2.5 μCi/ ml [^{3}H]thymidine in the presence of 10^{-5} M cold thymidine for periods from 1 to 6 hr, cells were solubilized with 1 ml 1 N NaOH and the DNA was precipitated with an equal volume of cold 20% TCA. The precipitates were trapped on glass-fiber filters and counted. The rate of DNA synthesis at a given cell density was obtained from a plot of [^{3}H]thymidine incorporated versus incorporation time.

Table 3
Mitotic Fraction of Cell Lines at Different Cell Densities

Cell density	Percent of cells in mitosis/hr[a]		
	3T3	*SV101*	*FlSV101*
Sparse	3.7	4.2	5.4
Partly confluent	0.08		0.48
Fully confluent	0.04	7.6	0.18
Residual activity[b]	1.1	180	3.3

[a] After exposure to 0.01 μg/ml Velban (Gibco) for 2 or 3 hr, attached cells were trypsinized, pooled with cells floating in the medium, centrifuged, swelled in 0.38% KCl, fixed in methanol-acetic acid (3:1) overnight, recentrifuged and spread onto clean, wet, cold slides. The slides were stained with Giemsa, dehydrated, mounted and examined at 400×. At least 1000 nuclei were counted for each determination. The counts were normalized to fraction in mitosis/hr.

[b] Mitotic index at confluence as percent of rate in sparse culture.

Alterations in DNA Content per Cell

All revertants isolated from SV101 have more chromosomes and more DNA per cell than the transformed parent line (Fig. 5). Using the Los Alamos Flow Microfluorometer, we have found that SV101 and 3T3 have, as expected for established cell lines, more DNA per cell than normal somatic mouse cells (Fig. 4). If primary mouse embryo fibroblasts are assumed to have twice the haploid amount of DNA (2c), then 3T3 and SV101 each have three times the haploid amount (3c) (Fig. 4). When serum and density revertants derived from SV101 were examined, all were found to contain more DNA than either 3T3 or SV101 (Fig. 4, 5). For example, FlSV101 density revertant cells had a DNA content equivalent to 6.5c (Fig. 4).

The average chromosome number per cell also increased in all revertants (Fig. 5). However the variation in chromosome number per cell was too great in these aneuploid lines to permit us to estimate whether the increase in chromosome number was equivalent to the increase in DNA for each line.

These increases in DNA and chromosomes are not merely an inadvertant consequence of the selection procedure per se. The lines aγ61, aγ12d, aγ256 survived negative selection with BrdU in agamma-depleted serum, but still grew in 1% calf serum and grew dense in 10% calf serum. These lines did not acquire any DNA or chromosome increase (Fig. 5). Also dense sublines derived from FlSV101 have regained the lower chromosome number characteristic of SV101 (Pollack, Wolman and Vogel 1970). Apparently the increases we observe are not nonspecific alterations but are specifically associated with the revertant phenotype.

Intracellular Concentration of Cyclic AMP

Cyclic AMP has been implicated as a possible mediator of cell growth (Otten et al. 1971; Sheppard 1972). To investigate the role that cAMP may play in the regulation of cell growth, we have measured intracellular cyclic AMP levels in

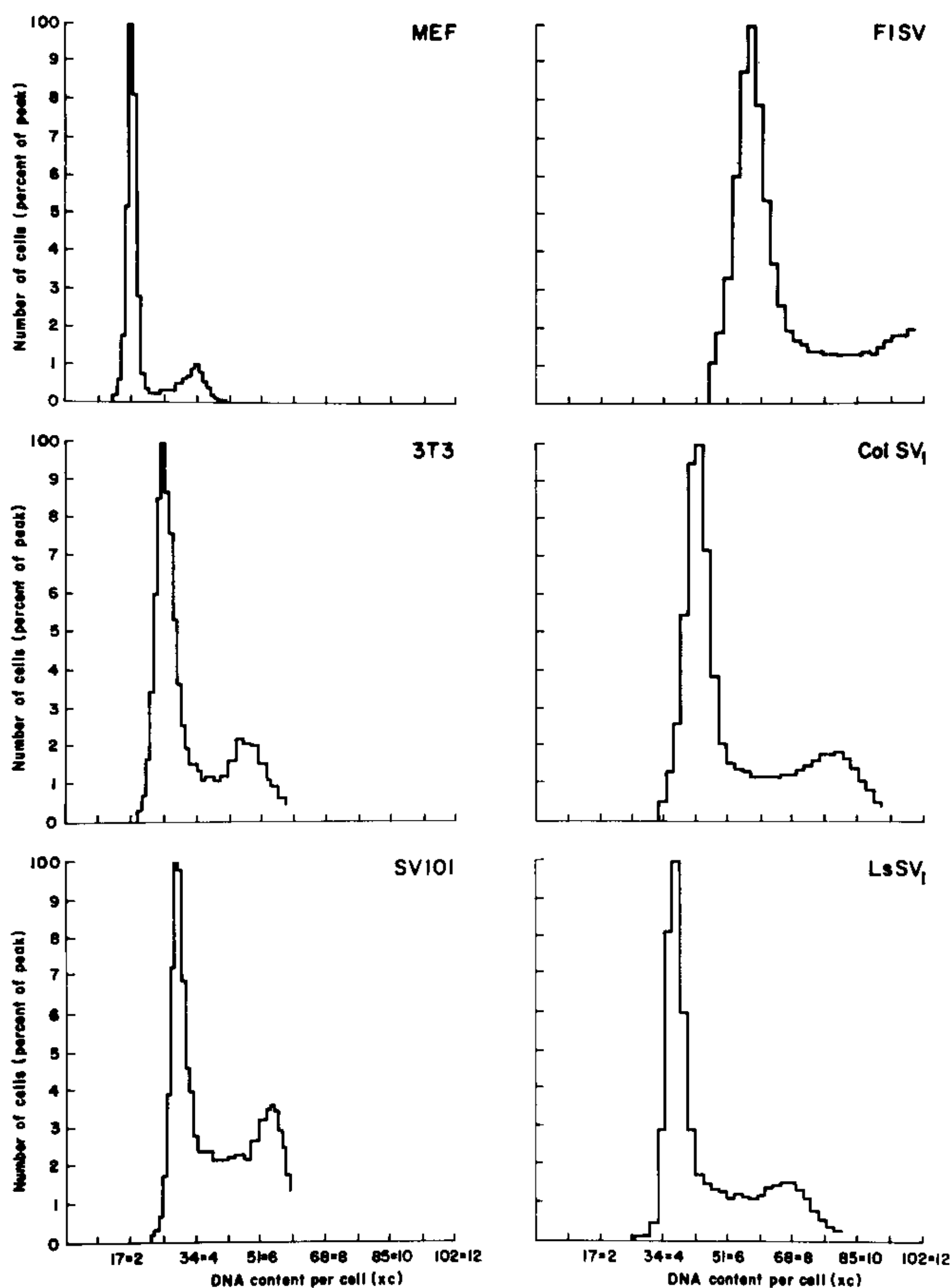


Figure 4

The distributions of DNA content (in arbitrary units) from Flow microfluorometry of growing cell lines. On the abscissa, the arbitrary scale is converted to multiples of the haploid amount (h) of mammalian cell DNA. For each line, the left-most peak represents cells in G_1. *MEF*, normal primary mouse embryo fibroblasts; *3T3*, normal established mouse cell line; *SV101*, SV40-transformed clone derived from 3T3; *FlSV*, density revertant derived by FdU selection from SV101; *ColSV*$_1$, density revertant derived by colchicine selection from SV101 (Vogel, Risser and Pollack 1973); *LsSV*$_1$, serum revertant derived by BrdU selection from SV101.

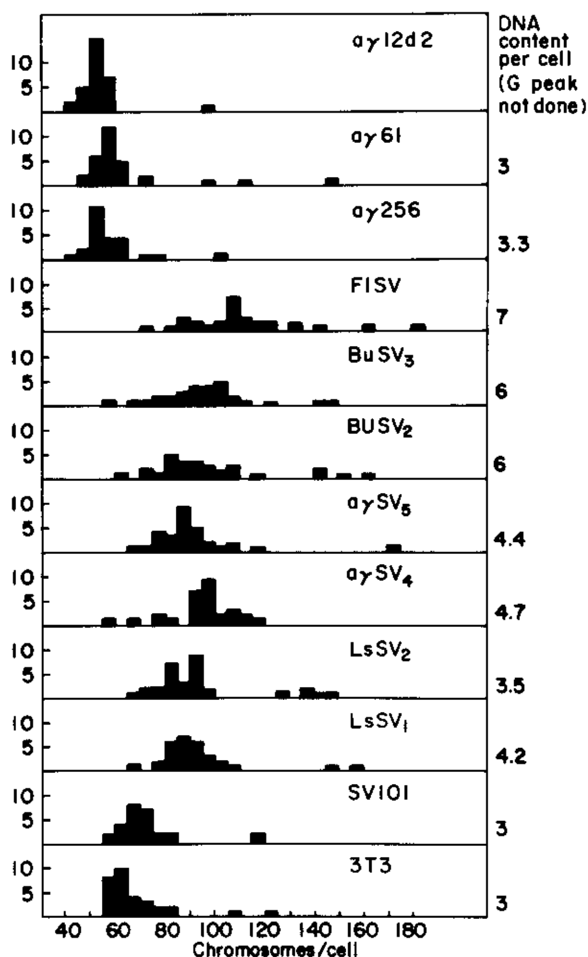


Figure 5
Chromosomes and DNA content per cell. aγ12d2, aγ61, and aγ256 are lines which survived the serum selection but can still grow in 1% calf serum. DNA content per cell expressed as a multiple of the haploid DNA content of primary mouse fibroblasts (see Fig. 4). From Vogel and Pollack (1973) with permission of *J. Cell. Physiol.*

three growth conditions: sparse culture in excess serum, confluence in excess serum, and sparse culture in low concentrations of serum. The first condition compares the cyclic AMP levels among cultures that are all growing at similar rates. The latter two conditions are restrictive for some lines but not others. Therefore they compare cells able to grow versus cells unable to grow.

Sparse Density in 10% Calf Serum

3T3, SV101 and all the revertants grow with approximately the same doubling time in 10% calf serum. Although the doubling times are similar, the intracellular cyclic AMP levels in these cells are not (Table 4). Normal 3T3 cells have a cAMP level twice that of SV101. This finding agrees with other published reports (Otten et al. 1971; Sheppard 1972). With the exception of the Aγ serum revertants, reversion in either the serum or density property is accompanied by reversion to a high cAMP concentration (Table 4).

Confluent Densities in 10% Calf Serum

3T3 and the density revertants cease to increase in cell number at confluent cell densities, but SV101 cells continue to grow. The cAMP concentration in confluent cultures of 3T3 has been reported to increase with increasing cell density (Otten et

Table 4
Cyclic AMP Concentrations in Sparse Cultures of Mouse Cell Lines in Excess or Restrictive Serum

	Line	*cAMP level (pmoles/mg protein)*[a]			
		10% calf serum		*1% calf serum*	
		range[b]	*avg*	*range*[b]	*avg*
Parent lines	3T3	20–28	25	75–100	83
	SV101	8–14	12	25–40	31
Serum revertants	LsSV1	17–22	20	52–70	68
	LsSV2	21–30	24	75–80	78
	aγSV4	10–15	13	60–75	67
	aγSV5	10–16	15	60–85	72
Density revertants	FlSV101	21–25	23	31–43	37
	BuSV2	17–25	21	30–46	34

[a] Cyclic AMP was determined by the Gilman assay.

[b] Four experiments, 4 determinations each, measured at 2 days after plating. Cell densities were 5×10^4–2×10^5 cells/plate, except SV101, which was 1×10^5–5×10^5.

al. 1971). When we determined cyclic AMP concentrations at different cell densities in 10% calf serum, we found not an increase, but a decrease in cyclic AMP at confluence (Table 5). This decrease occurred in all lines tested, even those that ceased to increase in cell number at confluence. Even the Aγ serum revertants, which might have been expected to show an increase in cAMP at confluence, maintained their low level.

Table 5
Cyclic AMP Concentration vs. Cell Density

Line	Cell density (cells/28-cm² dish)	*cAMP concentration* pmoles/mg protein	pmoles/10⁶ cells
3T3	2.5×10^5	21	12
	1.5×10^6	17	9
SV101	7.5×10^5	11	7
	1.5×10^7	8	1.5
LsSV2	2.5×10^5	21	13
	3.0×10^6	10	4
aγSV4	2.5×10^5	10	6
	3.0×10^6	6	2

Cells were seeded at 10^5 cells per 60-mm dish. cAMP was determined 2 days after plating. Upon reaching confluence the medium was changed and the cAMP level was determined 3 days later.

Sparse Density in 1% Calf Serum

This condition restricts the growth of the serum revertants and 3T3, but does not markedly affect the growth of SV101 or the density revertants. Cell lines unable to grow well in 1% calf serum showed a marked increase in cAMP concentration (Table 4). The concentration of cAMP in restricted lines exceeded 70 pmoles/mg protein, which was more than twice the concentration found in lines (such as SV101 and the density revertants) that grew in 1% calf serum (Table 4).

These data permit three conclusions. First, growing cultures maintain different levels of cAMP, and these levels are higher in lines that have the ability to regulate their growth at high cell density. Second, the process of density inhibition of cell growth does not lead to a further rise in cAMP in such cultures at confluence. Third, cells that are unable to grow in sparse culture in 1% calf serum because of an increased requirement for serum increase their cAMP concentration markedly; this increase correlates with the serum dependence, and does not correlate with the density inhibition, of these lines.

SV40 in Revertant Cell Lines

All of the revertants contain SV40 T antigen detectable by immunofluorescence (Table 6) and SV40-specific RNA (Table 7). The number of SV40 genomes per haploid amount of cell DNA was found to be the same in the density revertant FlSV101 as the parent SV101 line (Ozanne, Sharp and Sambrook 1973; Ozanne et al. 1973).

Most revertants yielded little or no infectious SV40 after fusion with permissive BSC-1 cells (Watkins and Dulbecco 1967). SV40 virus recovered after fusion was wild type with respect to its ability to transform 3T3 cells (Table 6). Mutant SV40, capable of growth on BSC-1 cells but defective in its ability to transform 3T3 cells, has not been rescued from any of the revertants.

Reinfection of the revertants with SV40 does not restore the high saturation density, nor does it confer the ability to grow in 1% calf serum (Table 6). However the revertants are retransformable by murine sarcoma virus, so they are capable of responding to an oncogenic virus (Table 6). This result extends the previous report of Renger (Renger 1972).

SV101 contains SV40 RNA sequences specific for 80% of the "early" strand of SV40 DNA and less than 10% of the "late" strand (Sambrook, Sharp and Keller 1972). The amount and strand specificity of SV40 RNA in the revertants are similar to those of the transformed parent (Table 7).

With one exception all variation in transcription seen among the revertants is within the range of variations seen among different SV40-transformed lines (Sambrook et al. 1972). The exception is in FlSV101, which contains less SV40-specific RNA than any of the other T antigen positive cell lines (Table 7). Despite this single exception, it is clear that reversion does not require a failure in transcription of SV40-specific DNA sequences.

DISCUSSION

The two classes of revertants, serum and density, demonstrate that it is possible to affect saturation density without altering the serum property, but that to alter the serum property is to simultaneously alter saturation density. A virus-transformed

Table 6
Rescue of SV40 from Revertant Lines

	Cell line	*T antigen*	*PFU/10⁶ mouse cells after 6 days*	*PFU/TFU*	*Retransformed by*	
					SV40	*MSV*
Parent lines	3T3	–	not done		+	+
	SV101	+	2000	1600	NT	NT
Serum revertants	LsSV1	+	0		–	NT
	LsSV2	+	0		NT	+
	aγSV4	+	0		–	+
	aγSV5	+	20	2000	–	NT
Density revertants	FlSV101	+	0		–	+
	BuSV2	+	0		NT	NT
	BuSV3	+	20	2000	–	NT

SV40 T antigen was assayed as immunofluorescence. Cells were fixed in cold methanol: acetone (1:2) for 10 min. The fixed cells were then stained with hamster anti-T antibody and counter-stained with fluorescein-conjugated goat anti-hamster antibody.

One million transformed or revertant cells were fused with 10^6 BSC-1 cells, plated and incubated for 6 days. The plates were then freeze-thawed twice, sonicated and plaqued on BSC-1 cells.

SV40 transformation was done according to Todaro et al. (1964) and MSV transformation was done according to Renger (1972).

Nt = not tested.

Table 7
Transcription of SV40 DNA in Mouse Cell Lines

	% DNA sequences present in RNA[a]	
	E strand	*L strand*
3T3	0	0
SV101	81	4
FlSV101	40[b]	5[b]
BuSV2	59	1
BuSV3	64	4
LsSV1	42	1
LsSV2	50	1
aγSV3	70	10
aγSV4	78	12
aγSV5	60	10

[a] Taken from Ozanne et al. (1973). Hybridization was done as described in Sambrook et al. (1972).

[b] With this line it was impossible to saturate the SV40 DNA with RNA.

line with a high saturation density and normal serum requirement has not yet been isolated.

Two conditions that restrict cell growth have been described: confluent cell density in 10% serum and sparse density in 1% serum. Cyclic AMP levels are clearly dependent on the serum concentration and reflect the ability of a line to grow in a given serum concentration (Table 4). Normal 3T3 cells and the serum revertants cannot grow in 1% calf serum and have very high cAMP levels in 1% calf serum, whereas SV101 and the density revertants can grow in 1% calf serum and display similar low cAMP levels in 1% calf serum.

Normal 3T3 cells maintain a low saturation density by stopping DNA synthesis and mitosis upon forming a confluent monolayer. The density revertant FlSV101 shows a density-dependent decrease in rate of DNA synthesis, in the fraction of cells synthesizing DNA, and in the fraction of cells in mitosis.

Although FlSV101 does respond to contact, the shut off of DNA synthesis is not as "tight" as in confluent 3T3 cells. We do not yet know how the serum revertants maintain a low saturation density in 10% calf serum.

Revertants contain SV40 T antigen, and the amount and strand specificity of the SV40-specific RNA in the revertants resemble those of the transformed parent. The revertant phenotypes therefore are not the result of the inability to transcribe the SV40 genome. The fact that nondefective SV40 can be rescued from the revertants implies that the reversion in transformed growth properties does not require all copies of any single SV40 gene in the cell to be defective.

Acknowledgments

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REFERENCES

Black, P. 1966. Transformation of mouse cell line 3T3 by SV40: Dose response relationship and correlation with SV40 tumor antigen production. *Virology* **28:**760.

Black, P., W. Rowe, H. Turner and R. Huebner. 1963. A specific complement-fixing antigen present in SV40 tumor and transformed cells. *Proc. Nat. Acad. Sci.* **50:**1148.

Culp, L. and P. Black. 1972. Contact inhibited revertant cell lines isolated from simian virus 40-transformed cells. III. Concanavelin A-selected revertant cells. *J. Virol.* **9:**611.

Culp, L., W. Grimes and P. Black. 1971. Contact inhibited revertant cell lines. I. Biologic, virologic and chemical properties. *J. Cell Biol.* **50:**682.

Dulbecco, R. 1970. Topoinhibition and serum requirement of transformed and untransformed cells. *Nature* **227:**802.

Eckhart, W., R. Dulbecco and M. Burger. 1971. Temperature-dependent surface changes in cells infected or transformed by a thermosensitive mutant of polyoma virus. *Proc. Nat. Acad. Sci.* **68:**283.

Holley, R. and J. Kiernan. 1968. Contact inhibition of cell division in 3T3 cells. *Proc. Nat. Acad. Sci.* **60:**300.

Jainchill, J. and G. Todaro. 1970. Stimulation of cell growth *in vitro* by serum with and without growth factor; relation to contact inhibition and viral transformation. *Exp. Cell Res.* **59:**137.

Otten, J., G. Johnson, and I. Pastan. 1971. Cyclic AMP levels in fibroblasts: Relationship to growth rate and contact inhibition of growth. *Biochem. Biophys. Res. Commun.* **44:**1192.

Ozanne, B. 1973. Variants of 3T3 cells transformed by SV40 that are resistant to concanavalin A. *J. Virol.* **12:**79.

Ozanne, B. and J. Sambrook. 1971. Isolation of lines of cells resistant to agglutination by concanavalin A from 3T3 cells transformed by SV40. *Lepetit Colloq. Biol. Med.* **2:**247.

Ozanne, B., P. Sharp and J. Sambrook. 1973. Transcription of simian virus 40. II. Hybridization of RNA extracted from different lines of transformed cells to the separated strands of SV40 DNA. *J. Virol.* **12:**90.

Ozanne, B., A. Vogel, P. Sharp, W. Keller and J. Sambrook. 1973. Transcription of SV40 DNA sequences in different transformed cell lines. *Lepetit Colloq. Biol. Med.* **4.** (in press).

Pollack, R. and A. Vogel. 1973. Isolation and characterization of revertant cell lines. II. Contact inhibition in a polyploid density revertant line derived from SV40 transformed 3T3 mouse cells. *J. Cell. Physiol.* **82:**93.

Pollack, R., H. Green and G. Todaro. 1968. Growth control in cultured cells: Selection of sublines with increased sensitivity to contact inhibition and decreased tumor-producing capacity. *Proc. Nat. Acad. Sci.* **60:**126.

Pollack, R., S. Wolman and A. Vogel. 1970. Reversion of virus transformed cell lines: Hyperploidy accompanies retention of viral genes. *Nature* **228:**967.

Renger, H. 1972. Temperature sensitive SV40 transformants retransformed by murine sarcoma virus at non-permissive temperature. *Nature New Biol.* **240:**19.

Sambrook, J., P. Sharp and W. Keller. 1972. Transcription of simian virus 40. I. Separation of the strands of SV40 DNA and hybridization of the separated strands to RNA extracted from lytically infected and transformed cells. *J. Mol. Biol.* **70:**57.

Scher, C. and W. Nelson-Rees. 1971. Direct isolation and characterization of "flat" SV40 transformed cells. *Nature New Biol.* **233:**263.

Seifert, W. and D. Paul. 1972. Levels of cyclic AMP in sparse and dense cultures of growing and quiescent 3T3 cells. *Nature New Biol.* **240:**281.

Sheppard, J. 1972. Differences in the cAMP levels in normal and transformed cells. *Nature* **236:**14.

Smith, H., C. Scher and G. Todaro. 1971. Induction of cell division in medium lacking serum growth factor by SV40. *Virology* **44:**359.

Stoker, M. 1968. Abortive transformation by polyoma virus. *Nature* **218:**234.

Todaro, G. and H. Green. 1963. Quantitative studies on the growth of mouse embryo cells in culture and their development into established lines. *J. Cell Biol.* **17:**299.

Todaro, G., H. Green and B. Goldberg. 1964. Transformation of properties of an established cell line by SV40 and polyoma virus. *Proc. Nat. Acad. Sci.* **51:**66.

Vogel, A. and R. Pollack. 1973. Isolation and characterization of revertant cell lines. IV. Direct selection of serum-revertant sublines of SV40-transformed 3T3 mouse cells. *J. Cell. Physiol.* (in press).

Vogel, A., R. Risser and R. Pollack. 1973. Isolation and characterization of revertant cell lines. III. Isolation of density-revertants of SV40-transformed 3T3 cells using colchicine. *J. Cell. Physiol.* (in press).

Watkins, J. and R. Dulbecco. 1967. Production of SV40 virus in heterokaryons of transformed and susceptible cells. *Proc. Nat. Acad. Sci.* **57:**1396.

Wyke, J. 1971. A method of isolating cells incapable of multiplication in suspension culture. *Exp. Cell. Res.* **66:**203.