

CONTACT-INHIBITED REVERTANT CELL LINES ISOLATED FROM SV40-TRANSFORMED CELLS

I. Biologic, Virologic, and Chemical Properties

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ABSTRACT

Two contact-inhibited "revertant" cell lines were isolated from an SV40-transformed mouse 3T3 cell line (SV-3T3) after exposure to 5-fluoro-2'-deoxyuridine. Revertant cells resembled 3T3 cells morphologically and grew to saturation densities which were similar to those of 3T3 cells; however, revertant cells readily formed both single and multinucleated giant cells in confluent cultures. SV40 virus was rescued from revertant cells by fusion with permissive monkey cells. The rescued virus transformed 3T3 cells with the same efficiency as wild type virus, and produced transformed colonies which were phenotypically similar to those produced by wild type virus. The revertant cells also resembled normal 3T3 cells in that they contained higher quantities of sialic acid than SV-3T3 cells. An inverse correlation was found between the saturation density of cells and their sialic acid content. Collagen content, however, of revertant cells was similar to that of SV-3T3 cells. The data presented suggest that the property of contact inhibition in revertant cells is related to the sialic acid content of the plasma membrane and that changes in sialic acid content of transformed cells are not directly specified by the viral genome.

INTRODUCTION

Wu et al. (1) and Meezan et al. (2) have reported differences in the neutral and amino sugar composition and glycoprotein distribution of various cell membranes derived from clones of 3T3 Swiss mouse cells and simian virus 40 (SV40)¹ trans-

formed 3T3 (SV-3T3) cells. Although the SV-3T3 cells lost contact inhibition of growth and grew to high saturation densities, it was not possible for these authors to determine whether the observed differences in the sugar and/or glycoprotein pattern could be correlated with the loss of contact inhibition. In addition, it could not be concluded that the presence of new viral genes in the transformed cells determined the sugar and glycoprotein changes, either directly or indirectly. In order to determine the relationship between the chemical composition of the plasma membrane, the presence of viral genes, and the loss of contact inhibition, it seemed important to isolate and study

¹ *Abbreviations used in this paper:* AGMK, African green monkey kidney cells; FUdR, 5-fluoro-2'-deoxyuridine; MEM × 4, Eagle's minimal essential medium supplemented with a fourfold concentration of vitamins and amino acids, 10% fetal calf serum, and antibiotics; PBS, phosphate-buffered saline; pfu, plaque-forming units; ST-3T3, spontaneously transformed 3T3 cells; SV-3T3, simian virus 40-transformed 3T3 cells; SV40, simian virus 40.

the properties of "revertant" cell lines, which are relatively contact inhibited, from virus-transformed cells (3-7).

We describe here some biologic, virologic, and chemical properties of cell lines isolated from the SV-3T3 clone analyzed by Wu et al. (1) and Meezan et al. (2). 5-Fluoro-2'-deoxyuridine (FUdR) was used as the selecting agent in a modification of the procedure described by Pollack et al. (3). In the following paper, ultrastructural studies of normal, transformed, and revertant cell lines will be presented.

MATERIALS AND METHODS

Cells

The origin and history of the normal 3T3 Swiss mouse cell line, the SV-3T3 cell line, and the spontaneously transformed (ST-3T3) cell lines have been described. The former cells were used between their 5th and 15th passages after cloning, whereas the SV-3T3 cells were utilized between their 50th and 80th passages after isolation (8). All cells were grown in 8- or 32-oz glass bottles in Eagle's minimal essential medium supplemented with a fourfold concentration of vitamins and amino acids, 10% fetal calf serum, and antibiotics (MEM \times 4). Saturation densities of cell lines were determined by inoculating 5×10^5 cells into plastic Petri dishes (50 mm diameter) and changing the medium every other day. Cell counts were determined on trypsinized cell suspensions daily for at least 4 days after confluence had been achieved. 3T3 cells were free of *Mycoplasma* contamination, while SV-3T3 cells and revertant cells were found to be contaminated with *Mycoplasma laidlawii*.²

Isolation of Revertants

A modification of the method of Pollack et al. (3) was utilized. 1×10^5 SV-3T3 cells (passage 53) were inoculated into 50-mm plastic Petri dishes. After 24 hr, the medium was removed and 5 ml of fresh medium containing 250 μ g of FUdR and 2500 μ g of uridine was added. After 48 hr in FUdR-containing medium, during which time the cells became very granular, the medium was removed. The sparse layer of cells remaining was washed twice with phosphate-

buffered saline, pH 7.2 (PBS), dispersed with trypsin, and the cells were passed to fresh dishes containing 5 ml of MEM \times 4 supplemented with 10% tryptose phosphate broth. This medium was replaced twice weekly for the following 3-5 wk. At that time colonies, generally 12-15 per dish, were isolated by surrounding them with steel cylinders 7 mm in diameter. Individual colonies were then dispersed with trypsin and passed. Two clones (F1A and F1E) were isolated by surrounding well-isolated cells with stainless steel cylinders by a modification of the method of Puck (9).

Virologic Procedures

The presence of the SV40-specific T antigen was determined by the indirect fluorescent antibody technique as described (10). SV40 virus was rescued from SV-3T3 and revertant cells by fusion with MA-134 cells, a continuous cell line derived from African green monkey kidney cells (AGMK), in the presence of Sendai fusion factor, as described by Burns and Black (11). After fusion, the cells were maintained in roller tubes and harvested after 4 and 10 days. Cell-free extracts were prepared and the virus was assayed by plaque formation on AGMK cells as described (11). Virus from revertant cells was passaged once in MA-134 monkey kidney cells and purified by concentration and treatment with trypsin and sodium deoxycholate as described (12). Wild type SV40 virus was purified as described (12).

Transformation assays were performed as described elsewhere (8). Approximately 3×10^8 plaque-forming units (pfu) of SV40 virus was adsorbed to 2×10^6 confluent 3T3 cells at 37°C in 10-cm plastic Petri dishes over a period of 2 hr; control plates were mock-infected with PBS. 15 ml of MEM \times 4 were then added to the dishes. After incubation for 4 hr, the medium was decanted, the cells were dispersed with trypsin, and the following concentrations of cells were plated into replicate 50-mm Petri dishes: 250, 500, 1000, 2000, 5000, and 2×10^4 . Cells also were inoculated into dishes containing cover slips, which were fixed and stained for T antigen after 24 hr in culture. Cultures were fixed with methanol and stained with Wright's stain 18 and 24 days after virus infection. Transformed foci and colonies were identified by criteria which have been described (8).

Chemical Determinations

SIALIC ACID: Sialic acid content of cells was determined by previously described methods (13). In this procedure, monolayers of confluent or sub-confluent cells were washed with 50 ml of solution A (0.8% NaCl, 0.05% KCl, 0.001 M KH_2PO_4 , pH 7.4) containing 100 mg/liter of MgSO_4 and CaCl_2 (buffer I). Cells were removed from the bottle in 25 ml of

² The properties of SV-3T3 and revertant cells which are compared in this paper probably are not a result of *Mycoplasma* contamination, since both cell types are contaminated with *Mycoplasma laidlawii*. Grimes (13) has found that *Mycoplasma* contamination has no effect upon the sialic acid content or sialyl transferase activities in 3T3 or SV-3T3 cells.

buffer I by scraping with a rubber policeman. The cell suspension was centrifuged at 600 *g* for 5 min. The resulting pellet was washed once by suspension in 25 ml of buffer I and re-sedimented. The pellet obtained was stored at -20°C .

For the determination of sialic acid, the frozen cell pellet was suspended in H_2O , and a 0.5 ml sample (containing 2–5 mg of protein) was adjusted to pH 1.0 by the addition of 4.5 *N* H_2SO_4 . The tubes were sealed and heated to 85°C for 1 hr. These hydrolysis conditions result in maximal release of sialic acid. After hydrolysis, duplicate 50- μl samples were assayed for protein by the Lowry procedure (14), and duplicate 0.1 ml samples were removed and assayed for sialic acid by a micro modification of the standard thio-barbituric acid assay (26). Absorbances were routinely read at 532 and 549 $m\mu$, and the contribution of chromophores absorbing at 532 $m\mu$ was eliminated as described by Warren (15).

COLLAGEN: For determination of the amounts of collagen produced in confluent 3T3, SV-3T3, and revertant cell lines, cells were grown to confluence in 4.5×14 -inch roller bottles in MEM $\times 4$ supplemented with 50 $\mu\text{g}/\text{ml}$ ascorbic acid and 1 $\mu\text{g}/\text{ml}$ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ as described by Green and Goldberg (16). The cells were maintained in culture for 48 hr after confluence had been achieved, washed once with PBS, scraped into 50 ml of PBS with a rubber policeman in order to minimize the loss of material in the extracellular matrices of cells (17), and pelleted by centrifugation at 600 *g* for 10 min in the PR-2 centrifuge. Pellets were dried overnight at 50°C and then hydrolyzed in 2.0 ml of 6 *N* HCl at 100°C overnight to hydrolyze proteins to amino acids. The level of collagen was determined by quantitating the amount of hydroxyproline on the UR-30 column of a Beckman amino acid analyzer. Amino acid determinations were performed by reaction with ninhydrin, measuring absorbances at 570 $m\mu$, and comparing them to absorbances produced with a leucine standard (18).

Materials

FUdR was donated by Hoffmann-La Roche, Inc., Nutley, N. J. The following materials were purchased: uridine from Nutritional Biochemicals Corporation, Cleveland, Ohio; sialic acid and L-leucine from Sigma Chemical Co., St. Louis, Mo.; primary AGMK cells, MA134 cells, and fetal calf serum from Microbiological Associates, Inc., Bethesda, Md.; fluorescein-conjugated goat anti-hamster serum from Hyland Laboratories, Los Angeles, Calif.; Wright's stain and ascorbic acid from Fisher Scientific Co., Pittsburgh, Pa.; Giemsa and Jenner stains from Harleco, Philadelphia, Pa.; and plastic Petri dishes from Falcon Plastics, Division of Bioquest, Oxnard, Calif.

RESULTS

Isolation of Revertant Cell Lines

One of the colonies that survived the exposure to FUdR described above contained larger cells which did not grow over one another, in contrast to the smaller SV-3T3 cells which grew into multi-layered colonies. This colony was isolated and two clones were established from it. These revertant clones (F1A and F1E) resembled 3T3 cells morphologically; both 3T3 and revertant lines contained cells which were large, pleomorphic, and polygonal or epithelioid in morphology (Fig. 1). The nuclei were round or ovoid and contained 5–10 nucleoli. Many multinucleated giant cells were present in the revertant cell lines. In addition, numerous, single-nucleated giant cells with very large nuclei containing 15–35 nucleoli were present. In contrast, SV-3T3 cells were smaller, and more triangular or spindle-shaped in morphology. The nuclei contained 5–10 nucleoli. Multinucleated giant cells were present but occurred much less frequently than in revertant cell lines.

In Fig. 2, the growth curves of 3T3, SV-3T3 and the revertant cell lines are compared. The revertant cell lines grew to a saturation density of $1.5\text{--}2.5 \times 10^6$ cells per Petri dish, which was similar to that of 3T3 cells, which grew to a saturation density of $8 \times 10^5\text{--}1 \times 10^6$ cells per dish. SV-3T3 cells grew to saturation densities which were approximately 10-fold higher. In contrast to SV-3T3 cells, both revertant and normal cells did not increase in number after confluence had been achieved. Thus, revertant cells display contact-inhibition of growth although they achieve saturation densities slightly higher than those of 3T3 cells. Similar properties were described for revertants isolated by Pollack et al. by the same FUdR selection procedure (3).

In an attempt to isolate a revertant cell line which was as contact inhibited as the control 3T3 cell line, a second treatment of F1E revertant cells with FUdR as described above was carried out. Colonies which were isolated after such treatment had neither lower saturation densities nor unique morphologies when compared with F1E cells.

Virologic Studies

PRESENCE OF T ANTIGEN: The SV40 T antigen, detected by fluorescent antibody tests,

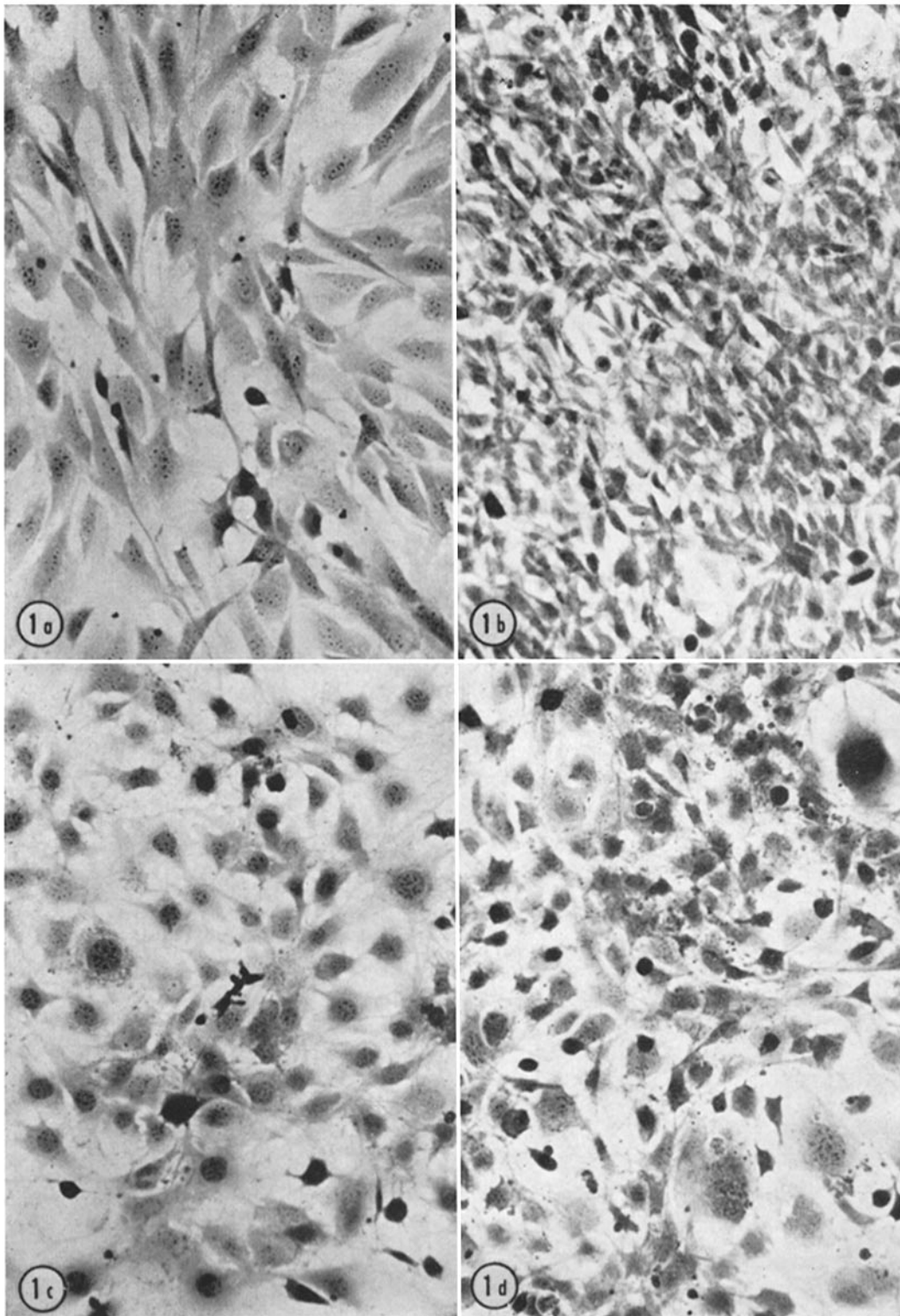


FIGURE 1 Morphology of normal, transformed, and revertant cell lines. (a) Confluent 3T3 cells; (b) hyperconfluent SV-3T3 cells; (c) confluent revertant F1A cells; (d) confluent revertant F1E cells. Cells were stained with combination Giemsa-Jenner blood stains 48 hr after confluence of the cultures had been achieved. $\times 175$.

was present in nearly all nuclei of the F1A and F1E revertant cell lines (Table I). The intensity of the staining of revertant cells was approximately equivalent to the intensity of staining with SV-3T3

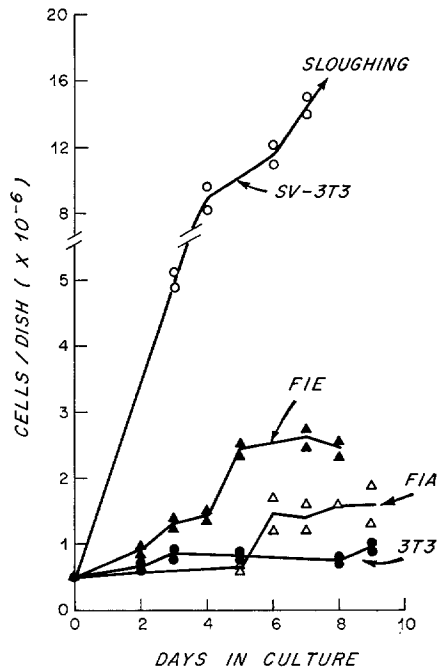


FIGURE 2 Saturation densities of normal, transformed, and revertant cell lines. Duplicate dishes (50-mm plastic Petri dishes) were treated as described in the text to determine the total numbers of cells per dish. 3T3 cells (●—●); SV-3T3 cells (○—○); revertant F1A cells (△—△); and revertant F1E cells (▲—▲).

cells. Thus, revertant cells retain the ability of transformed cells to express at least one virus-induced function—T antigen.

VIRUS RESCUE: No infectious SV40 virus was recovered from frozen and thawed, sonicated, cell-free extracts of the two revertant cell lines examined. The extracts were derived from approximately 5×10^6 cells in 1.0 ml of PBS, of which 0.2 ml was adsorbed to each AGMK monolayer; reconstruction experiments have demonstrated that 1–5 pfu of SV40 can be recovered when added to such extracts.

Cell fusion studies were carried out with revertant F1E cells (at the fifth passage level) or SV-3T3 cells and MA134 cells in the presence of Sendai fusion factor as described in the Materials and Methods section. The results are shown in Table I. Extracts prepared from cultures maintained for 4 days after fusion of SV-3T3 or revertant cells with indicator cells contained no infectious virus; extracts prepared from cultures maintained for 10 days after fusion contained approximately the same amount of infectious virus. The virus recovered from revertant cells produced cytopathogenic effects in MA134 or AGMK cells which were similar to those produced by both wild type virus and virus rescued from SV-3T3 cells. Thus, revertant cells contain the entire SV40 genome (s).

TRANSFORMATION POTENTIAL OF RESCUED VIRUS FROM REVERTANT CELLS: One explanation for the presence of contact-inhibited cells in a population of virus-transformed cells is

TABLE I
Properties of Revertant Cells

	Saturation density ($\times 10^{-6}$)*	T antigen	SV40 virus†		Giant cells	Morphology‡
			4 day	10 day		
3T3	1	—	—	—	—	Large polygonal
SV-3T3	20	+	—	+	+	Small, polygonal, spindle shaped
Revertant F1A	1.6	+	+	+	+++	Large polygonal
Revertant F1E	2.5	+	—	+	+++	Large polygonal

* Cells per 50 mm plastic Petri dish.

† Rescued from cells by fusion with indicator MA134 cells in the presence of Sendai fusion factor. After fusion, the cells were maintained for 4 or 10 days in culture. Extracts were then prepared and assayed for virus content by plaque formation (see text).

‡ See Fig. 1.

|| The following quantities of virus were rescued: 1.5×10^3 pfu from revertant F1E extracts, and 3.2×10^3 pfu from SV-3T3 extracts. The extracts were prepared from 1×10^6 revertant or SV-3T3 cells, 3×10^6 MA 134 cells and Sendai fusion factor as above.

the presence of unique viral genomes in a small proportion of cells which permit them to be contact-inhibited. If viral genes are responsible for this phenomenon in revertant cells, one might expect cells transformed by virus rescued from revertant cells to be contact inhibited. To test this hypothesis, virus which had been rescued from revertant cells was used to transform 3T3 cells, and the transformation frequencies of this virus and wild type SV40 virus were compared. The data in Table II indicate that the transformation frequencies with either wild type virus or revertant virus are similar. After transformed colonies were evident by morphological criteria, 17 colonies were selected randomly from plates exposed to revertant virus. These colonies were isolated, grown to mass cultures, and characterized both for presence of T-antigen, as an indication of transformation, and for their saturation density

TABLE II
Transformation of 3T3 Cells with Virus Rescued from Revertant Cells

	T-antigen positive*	Transformation frequency †	pfu§/tfu
	%	%	
SV40 (wild type)	11.9	1.0	1.50×10^4
SV40 (revertant)	14.0	1.1	1.36×10^4

* Percentage of cells containing T antigen 24 hr after infection.

† Number of stained transformed foci divided by number of cells plated $\times 100$ (t cell). Each number calculated by averaging frequencies of 10 plates. Approximately 3.0×10^8 pfu of the appropriate virus was used to infect 2×10^6 3T3 cells. Since the plating efficiency was approximately 10% (as determined in plates in which colonies were present), the transformation frequency, which can also be expressed as the percentage of transformed colonies divided by the total number of colonies present on the dish $\times 100$, is 10–11% (t col). This corresponds to the number of cells containing T antigen 24 hr after infection – 12–14% which presumably yielded the transformed colonies. In calculations of t col, it was assumed that cells plated at approximately the same efficiencies for all the concentrations employed (see reference 8).

§ Ratio of plaque-forming units of virus to transformation-forming units of virus.

|| Virus rescued from revertant F1E cells and passaged once through MA134 cells.

to determine if any transformants are contact inhibited. All the colonies which stained positively for T antigen grew to much higher saturation densities than revertant cells and displayed the morphology characteristic of 3T3 cells transformed by SV40 virus (Table III). Thus, virus rescued from revertant cells does not yield 3T3 transformants which are contact inhibited or which have the morphology of revertant cells; the presence of a unique virus population which was rescuable, therefore, is probably not responsible for the contact inhibition of growth found in revertant cells.

RETRANSFORMATION OF REVERTANT CELLS: Attempts were made to retransform revertant F1E cells with SV40 virus at a multiplicity of infection of 3000 pfu per cell. No morphologically transformed colonies were detected in approximately 6×10^4 cells plated (see Materials and Methods). These data indicate that revertant cells display some resistance to retransformation. We have preliminary evidence that revertant cells are as permeable to radioactivity-labeled, purified SV40 virus as normal 3T3 cells;

TABLE III
*Properties of Colonies Originating from Cells Infected with Revertant Virus**

Colony number	Presence of T antigen	Saturation density ($\times 10^{-6}$ cells per dish)
37	+	7.0 ‡
39	+	7.3 ‡
42	+	7.4 ‡
43	+	8.1 ‡
44	+	7.0 ‡
45	+	11.0 ‡
3T3 cells§	–	1.5
Revertant F1E	+	2.5

* Colonies selected randomly from 3T3 cells infected with SV40 virus rescued from revertant cells; see Materials and Methods for details.

‡ At the indicated saturation densities (5–10 days in culture) some sloughing occurred and the cultures could no longer be maintained.

§ Nontransformed 3T3 cells of the same passage level as used for this experiment. The remaining 11 colonies, which did not contain T antigen, grew to saturation densities of approximately $1.5\text{--}2 \times 10^6$ cells per dish.

|| Revertant cells from which SV40 virus was rescued.

these data suggest that the failure to transform the revertant cell is not due to an adsorption block.

Chemical Properties

SIALIC ACID CONTENT: Measurements of sialic acid contents of 3T3 cells (Table IV) confirm observations of previous investigators that the sialic acid content is reduced in cells transformed by oncogenic DNA viruses (1, 13, 27). Furthermore, a comparison of 3T3 and SV-3T3 cells with spontaneously transformed 3T3 cells (ST-3T3) and revertants of SV-3T3 cells shows that the former cells (ST-3T3) have sialic acid contents similar to those of SV-3T3 cells, while the latter cells (revertants) have sialic acid contents similar to those of normal cells. Thus, when normal cells lose the ability to become contact inhibited, whether by spontaneous or oncogenic virus transformation, the sialic acid content decreases; revertants of SV40 virus-transformed cells, which have regained the ability to be contact inhibited, have a relatively increased sialic acid content. The data of Fig. 3 show this direct correlation between cell sialic-acid content and saturation density. If

sialic acid content had been expressed as a function of surface membrane area, the differences in amount of sialic acid between the cell lines would have been even greater. These same relationships in sialic acid content were found in confluent or subconfluent cultures of the three cell types.

COLLAGEN CONTENT: Green and Goldberg (16) have determined that confluent 3T3 cells produce measurable amounts of collagen. Conversely, 3T3 cells transformed by SV40 virus have diminished collagen levels. It was of interest to determine whether revertant cells may have regained the property of 3T3 cells to produce higher levels of collagen. Collagen levels of the various cell lines were compared by determining the ratio of hydroxyproline in acid hydrolysates (see Materials and Methods section) of whole cell sheets to the amount of total amino acids (see Table V). The data indicate that SV-3T3 cells

TABLE IV
Sialic Acid Content of Various Cell Lines

Cell line	μg of sialic* acid/mg protein	Per cent	Saturation density ($\times 10^{-6}$ cells per 50 mm dish)
3T3	$5.0 \pm 0.6\ddagger$	100	1.0
ST-3T3 I	$3.4 \pm 0.2\§$	68	6
ST-3T3 II	$3.3 \pm 0.0\§$	66	6
SV-3T3	$3.0 \pm 0.3\ddagger$	60	>20
Revertant SV-3T3 (F1E)	$4.4 \pm 0.6\§$	88	2.5
Revertant SV-3T3 (F1A)	$4.8 \pm 0.3\§$	96	1.6

* Two to four determinations were carried out for each experiment.

‡ Average of four experiments.

§ Average of two experiments.

Description of the cell lines and details of sialic acid-saturation density measurements are found in Materials and Methods section. Each sialic acid value is the average of two to four measurements. The " \pm " value is the difference between the average sialic acid content and the maximum deviation from the average. For the sake of comparisons, the sialic acid content of normal 3T3 cells was assigned an arbitrary value of 100%.

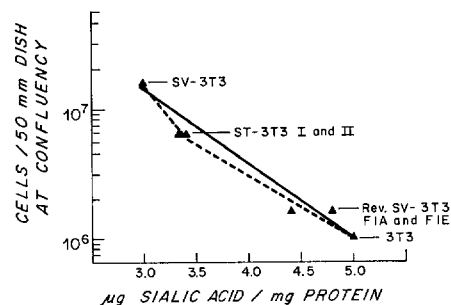


FIGURE 3 Sialic acid content and saturation densities of normal, transformed, and revertant cell lines. The cell saturation densities and sialic acid content are from the data of Table IV.

TABLE V
Hydroxyproline Content of Various Cell Lines*

Cell type	$\frac{\mu\text{moles hydroxyproline}\ddagger}{\mu\text{moles of amino acids}\§}$
3T3	19.0×10^{-4}
SV-3T3	2.6×10^{-4}
Revertant F1A	1.1×10^{-4}

* Cells grown and treated as described in the Materials and Methods section.

‡ Hydroxyproline was determined after acid hydrolysis by fractionation on the UR-30 column of the Beckman amino acid analyzer.

§ Total amino acid content of acid hydrolysates determined by assay with ninhydrin using a leucine standard (18).

do indeed produce much less collagen than confluent 3T3 cells, confirming the results of Green and Goldberg (16). Similarly, revertant F1E cells do not produce large quantities of collagen and, therefore, resemble SV-3T3 cells in this respect.

DISCUSSION

Two contact-inhibited, virus-transformed cell lines have been isolated by the FUdR selection technique described by Pollack et al. (3). The revertants were isolated after only one FUdR treatment, instead of the two exposures to FUdR required by the above authors (3); exposure of the revertant cells for a second time to FUdR did not result in cell lines with lower saturation densities. Only one flat-variant colony was found after treatment of 10^5 cells, indicating that this type occurs at a very low frequency in transformed cell cultures.

The origin of revertant cells in transformed cell cultures is not clear. Since FUdR inhibits DNA biosynthesis by preventing the formation of thymidine nucleotides which are precursors of DNA, it is possible that altered DNA metabolism has allowed mutagenic events to take place which give rise to a cell of this phenotype. On the other hand, SV-3T3 cultures may give rise to contact-inhibited cells at a very low frequency during cell division by segregation of unique genetic information. Exposure to FUdR might then act as a selection agent, as postulated by Pollack et al. (3).

These revertant cells resemble untransformed 3T3 cells in that they are larger, more polygonal, and have low saturation densities. A striking tendency of revertant cells to form both multinucleated and single-nucleated giant cells was observed. Such fusion of cells into syncytions with subsequent mixing of their chromosomes may explain the higher numbers of chromosomes which have been reported in revertant cells (20).³ There is some evidence that single-nucleated giant cells have a selective advantage in tissue culture (21). The reason for the enhanced fusibility of the revertant cells is not known; this phenomenon may be an indication of unique chemical properties in the surface membranes of these cells which permits fusion to occur readily. An alternative possibility that cannot be excluded is that the polyploid cells arise by defective cytokinesis.

The virus which was rescued from revertant

³ Pollack, R. E. Personal communication.

cells by fusion with permissive cells was similar to wild type virus in that it caused the typical cytopathic effects in monkey kidney cells and produced a similar frequency of transformation of 3T3 cells. The transformed colonies all contained SV40 T-antigen, had the typical morphology of SV-3T3 cells, and grew to high saturation densities. These studies indicate that the contact inhibition of revertant cells is probably not the result of the expression of unique viral genomes in these cells, although the presence of such genomes which were not rescued cannot be ruled out from our studies. The presence of the viral genomes may, however, be determining the resistance of revertant cells to superinfection which was indicated by our studies. The studies presented also indicate that the same yield of virus was rescued from SV-3T3 and revertant cells. Although this suggests that the same number of genomes are present in both types of cells, it is also possible that revertant cells contain fewer genomes per cell but that rescue is more efficient in revertant cell cultures because of their greater fusibility with indicator cells. However, it has not been established that the number of viral genomes in transformed virogenic cell lines can be directly correlated with the ease of rescue (22).

The evidence presented further suggests that the virus does not directly specify the changes in sialic acid content of transformed cells; it is also possible that host-cell genetic factors might be operative in determining sialic acid content of cell lines. Both Wu et al. (1) and Grimes (13) have determined that the sialic acid composition of SV-3T3 cells is approximately 40-50% lower than that of 3T3 cells per amount of cellular protein. In this study, we find that both revertant cell lines, each of which contains the complete SV40 genome(s), have regained the higher level of sialic acid characteristic of 3T3 cells. This suggests that the level of sialic acid may be an important factor in determining contact inhibition of growth; furthermore, an inverse relationship was found between the level of sialic acid and the saturation density that these cells achieve in culture. Spontaneously transformed 3T3 cells, which are not contact inhibited and do not contain viral genetic information, also show such a correlation between sialic acid content and saturation density. It is not known whether the changes observed occur for sialyl-containing glycolipids and/or sialyl glycoproteins.

Results from experiments measuring sialic acid content of normal, ST-3T3, SV-3T3, and revertant cells are consistent with the hypothesis that the sialic acid content of the plasma membrane may play an essential role in regulating normal cell-to-cell interaction. These sialic acid differences are presumably important in surface membrane chemistry and physiology, since the vast majority of cellular sialic acid is found in cell membranes (23, 24) and, quite possibly, surface membranes (25). Furthermore, many studies have shown that the absence of sialic acid results in disturbances of cell-to-cell relationships. For example, normal cell-to-cell aggregation of embryonic cells is dependent on the presence of sialic acid (19).

The decrease in sialic acid content seen with increasing saturation density may be paralleled by a similar decrease in other glycoprotein sugars. Wu et al. (1) have shown that many sugars, including sialic acid, were decreased in SV40 virus-transformed 3T3 cells. Whether sialic acid per se, or a large moiety containing sialic acid, either sialyl glycoprotein or sialyl glycolipid, is of importance in regulating normal cell-to-cell interaction requires additional study.

Revertant cell cultures did not contain greater quantities of collagen per amount of cellular protein than SV-3T3 cells. This would suggest that collagen production is not necessarily linked to the large, polygonal morphologies which characterize 3T3 and revertant cells, nor to their contact inhibition of growth.

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