Variants of Simian Virus 40-Transformed 3T3 Cells That Are Resistant to Concanavalin A

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By treating populations of simian virus 40 (SV40)-transformed 3T3 cells with concanavalin A, variants have been isolated which are resistant to the killing action of the lectin. The variants (i) resemble 3T3 cells morphologically and in some of their growth characteristics; (ii) are not agglutinated by high concentrations of concanavalin A or wheat germ agglutinin, but can be rendered agglutinable by treatment with low concentrations of trypsin; (iii) bind the same number of concanavalin A molecules as 3T3 or SV3T3 cells; (iv) cannot be transformed by SV40 and are resistant to focus formation after infection with murine sarcoma virus; (v) contain SV40-specific T antigen and RNA and; (vi) yield wild-type SV40 virus after heterokaryon formation with BS-C-1 cells.

The small DNA viruses, polyoma and simian virus 40 (SV40), cause tumors after injection into susceptible animals and transform a variety of cells in vitro. After transformation the cells assume a new set of heritable characteristics and contain one or more copies of viral DNA (11, 26, 31, 39) covalently linked to the host genetic material (33). Some of the viral DNA sequences are transcribed into RNA (4, 17, 26, 31) and virus-specific antigens can be detected (5, 6, 19, 20). In the case of cells transformed by polyoma virus, it seems that the expression of the viral genome is necessary to maintain at least some of the phenotypic characteristics of transformed cells (9, 10). With SV40, however, the relative contributions of the virus and the host cell to the transformed state remain obscure.

One approach to this problem has been to isolate variants of SV40-transformed cells which have regained the phenotype of normal cells. By using selection methods which exploit specific differences between normal and transformed cells, it is possible to obtain from populations of transformed cells, variant clones that have regained growth control either partially or completely. For example, Pollack, Green, and Todaro (28) used 5'-fluorodeoxyuridine to kill the dividing cells in dense cultures of 3T3 cells transformed by SV40 (SV3T3 cells). A small fraction of the surviving cells had stopped growing before the drug was added, presumably because they had recovered partial growth control. Renger and Basilico (30) extended this

technique to isolate variant cells which display the transformed phenotype when grown at 32 C but which resemble normal cells in the growth pattern at 39 C. Other groups (8, 24, 40) have used the differential effects of lectins on normal and transformed cells to isolate "revertant" lines of SV3T3 cells. The lectin concanavalin A (con A) distinguishes between normal and transformed cells in at least two ways—the transformed cells agglutinate in concentrations of con A which do not affect normal cells (15), and the transformed cells are preferentially killed (8, 24, 34, 40). The basis for these differential responses is unknown. However, they do not seem to result from the exposure of a large number of new or latent binding sites for the lectin on the surface of the transformed cells since both sorts of cells bind virtually equal numbers of con A molecules (3, 6, 25). Recently, several groups (21, 22) have demonstrated a reorganization of the existing binding sites after transformation. Nicolson argues that this alteration in the topology of the lectin-binding sites is important for the different interactions of normal and transformed cells with con A. Regardless of the mode of action of con A, there seems to be an inverse relationship between the degree of growth control exhibited by different cell lines and their susceptibility to lectin-mediated agglutination (28) and killing. We have taken advantage of the selective toxicity of con A for transformed cells to isolate lines of SV3T3 cells which are resistant to the lectin. This paper describes the properties of these

lines. A preliminary report of part of this work has already appeared (24).

MATERIALS AND METHODS

Cells. Swiss 3T3 cells (obtained from R. Pollack, Cold Spring Harbor Laboratory) and SV40-transformed Swiss 3T3 cells (obtained from W. Eckhart, Salk Institute) were grown in Dulbecco's modification of Eagle's medium, supplemented with 10% calf serum. BS-C-1 cells (14) (obtained from T. Benjamin, Public Health Laboratory of New York City) and MA-134 cells (obtained from J. Pagano, University of North Carolina) were grown in Dulbecco's modification of Eagle's medium supplemented with 10% fetal bovine serum. All cell lines were grown routinely on plastic tissue culture dishes (Nunc) at 37°C in 5% CO₂.

Viruses. A stock lysate of SV40 strain 777 (12) was grown on BS-C-1 cells. The Rauscher strain of murine sarcoma/leukemia virus (MSV) was a gift from S. Aaronson, National Institutes of Health. Inactivated Sendai virus was supplied by Hayden Coon, National Institutes of Health.

Growth curves. Cells were inoculated at a density of 10⁴ cells/cm² onto petri dishes in Dulbecco's modification of Eagle's medium containing 10% serum. The medium was changed every 3 days. At intervals after inoculation the cells were removed from the plate with trypsin and were counted in a Coulter counter. In order to determine the capacity of cells to grow in low concentrations of serum, the cells were seeded at 10⁴ cells/cm² in medium containing 10% calf serum and were incubated at 37°C for 4 h to allow the cells to attach to the petri dishes. The medium was then removed from the plate and the cells were washed three times with phosphate-buffered saline (PBS). Medium containing 1% calf serum was added and the cells were incubated at 37°C. Medium was changed every 3 days. The number of cells per square centimeter was determined as described above.

T antigen. T antigen was determined by immunofluorescence using fluorescent antisera purchased from Flow Laboratories, Rockville, Md.

Virus rescue and heterokaryon formation. Virus rescue and heterokaryon formation were performed as described by Watkins and Dulbecco (38).

Infectivity assays. (i) SV40: plaque assays for SV40 were carried out as described by Kimura and Dulbecco (18). (ii) MSV: focus-forming assays for MSV were performed as described by Aaronson, Jainchill, and Todaro (1).

Transformation assays with SV40. Transformation assays were carried out as described by Aaronson and Todaro (2).

Agglutination assays. Agglutination assays for con A and wheat germ agglutinin were carried out as described elsewhere (25). Con A (3 × crystallized in saturated NaCl) was purchased from Miles-Yeda Laboratories, Israel. Wheat germ agglutinin was purified from wheat germ lipase (Worthington Biochemicals, Freehold, N.J.) as described by Ozanne and Sambrook (25).

Binding of radioactive con A to cells. The preparation of ¹²⁵I-labeled con A and the details of the binding assay have been described previously (25).

Rate of DNA synthesis determination. The rate of DNA synthesis was measured by following the conversion of ³H-thymidine into acid-insoluble material. Cells were seeded at 2.5 × 10⁴ cells/cm², and growth was followed by counting the cells in a Coulter counter daily. Replicate cultures were pulse-labeled with ³H-thymidine at a final concentration of 1 μCi/ml in 2.5 × 10⁻⁵ M thymidine. Under these conditions of labeling, there is linear conversion of radioactivity into acid-insoluble material for periods up to 5 h. After 4 h the label was removed and the cells were washed three times with PBS. Then the cells were dissolved in 0.5 ml of 1 M NaOH. The acid-insoluble material precipitated by the addition of 5 ml of cold 5% trichloroacetic acid was collected on filters (GFC Whatman) and counted in a liquid scintillation counter.

RESULTS

Isolation of con A-resistant SV3T3 cells. A recently cloned line of SV3T3 cells was grown to a density of 3 × 10⁴ cells/cm², and con A was added directly to the medium to a concentration of 300 μg/ml. After incubation for 24 h at 37°C, most of the cells had rounded up and detached from the surface of the petri dishes. These cells were removed by repeated washing with PBS. Fresh medium was then added and the surviving cells (about 1 in 10⁵) were allowed to grow to form colonies. Two weeks after the initial treatment with con A, the colonies were examined by phase-contrast microscopy, and approximately one in twenty of the colonies observed displayed a "flat" morphology reminiscent of 3T3 cells. Sixty-three of these colonies were isolated, grown up, and treated with con A. Fifty-four of the clones had lost their flat morphology and were as sensitive to the killing effects of con A as the parent SV3T3 cells. Nine of the clones, however, remained flat and were clearly more resistant than the parent cells during two more rounds of con A treatment.

Three of the nine con A-resistant SV3T3 clones, CA³⁰, CA³², and CA⁴¹, were characterized further. These variants have been carried continuously in cell culture for 12 months in the absence of con A without significant variations in their colony morphology, their saturation density, or their resistance to con A. However, SV3T3-like cells occasionally appeared during prolonged passage of the cells in the absence of the lectin. When this occurred, the cell lines were recloned in the absence of con A. The morphology and saturation density of the variants were not altered after subcloning.

Growth properties. In culture, 3T3 cells grow until they form a monolayer and then stop growing completely or grow at a much reduced rate. Given the same conditions, SV3T3 cells

continue to divide, form multilayers, and attain cell densities 10 to 20 times those reached by 3T3 cells. The con A-resistant variants resemble 3T3 cells morphologically (Fig. 1), and the following experiments show they also possess growth properties similar to those of 3T3 cells.

(i) The data presented in Table 1 and Fig. 2a

show that CA^r32 and CA^r41 reach final saturation densities only slightly higher than that of 3T3 cells, whereas CA^r30 grows to a final density approximately twice that of 3T3 cells. If the medium is changed after the cells have reached saturation density, there is a small increase in cell number during the next 24 to 48

FIG. 1. Photographs of the cell line 3T3 (b), CA^r30 (d), CA^r32 (c), and CA^r41 (e), taken 2 days after the cells had reached their saturation densities; magnification of $\times 200$. The density of the SV3T3 (a) cells was 10^5 cells/cm².

TABLE 1. *DNA synthesis*

Cell line	Saturation density ^a (10 ⁴ cells/cm ²)	Sparse culture ^b		Confluent culture ^c		% Log-phase rate ^d of incorporation
		10 ⁴ cells/cm ²	Counts/min per h per 10 ⁴ cells	10 ⁴ cells/cm ²	Counts/min per h per 10 ⁴ cells	
3T3	8.5	5.2	127	8.9	1.3	1
SV3T3	76	7.1	91	59	73	65.8
CA*30	16	6.4	154	23	20.6	13
CA*32	10	5.8	131	11	26.7	20.5
CA*41	10.7	6.6	189	13	52.3	27.5

^a Saturation density was computed from Fig. 2a after there was no increase in cell number for two successive 24-h periods.

^b Sparse culture: cells were seeded at 2.5×10^4 cells/cm² and pulse-labeled with ³H-thymidine as described in Materials and Methods during log-phase growth.

^c Confluent culture: cells were seeded at 2.5×10^4 cells/cm² and allowed to grow until they reached their saturation densities; then the medium was changed, and 48 h later labeling was performed as in Materials and Methods.

^d Percent log-phase rate of DNA synthesis was computed as follows: (counts/min per hour per 10⁴ cells sparse culture)/(counts/min per hour per 10⁴ cells confluent culture) $\times$ 100.

h, after which the number of cells per unit of area remains unchanged (Fig. 2).

(ii) When large numbers of cells are inoculated onto petri dishes so that monolayers are formed after one round of cell division, neither 3T3 nor the con A-resistant SV3T3 variants increase in cell number after 24 to 48 h. Furthermore, changing the medium on these cultures 96 h after inoculation again results in only a slight increase in cell numbers over the next 48 h (Fig. 2c). SV3T3 cells, on the other hand, continue growing and reach a saturation density 10 times higher than that attained by 3T3 cells.

The conclusion from these experiments is that the con A-resistant cells, like 3T3 cells, do not form multilayers; and they have saturation densities which closely resemble those of untransformed cells.

Serum requirement. Cells transformed by SV40 require less serum to sustain growth than do untransformed cells (13, 16). To test whether the con A-resistant SV3T3 cells required high concentrations of serum in order to divide, the cells were grown in medium containing 1% calf serum. The number of cells in the culture was determined at intervals thereafter. As seen in Fig. 3, both the con A-resistant variants and SV3T3 cells grew in 1% serum; 3T3 cells, however, failed to divide. So even though the con A-resistant variants behave like 3T3 cells in showing a density-dependent inhibition of division, they resemble SV3T3 cells in their serum requirement. Thus the con A-resistant cells are another example of a class of cells already reported in the literature (36; A. Voge and R. Pollack, personal communication) whose behavior clearly establishes that it is possible for a

cell line to have a low serum requirement and a low saturation density.

DNA synthesis. Confluent monolayers of 3T3 cells synthesize DNA at only 1% of the rate shown by cells in the logarithmic phase of growth. Confluent SV3T3 cells, on the other hand, continue to synthesize DNA at a rate almost equivalent to that of subconfluent cells. The rate of DNA synthesis in confluent monolayers of con A-resistant SV3T3 cells was measured by determining the amount of ³H-thymidine incorporated into acid-precipitable material during a 4-h pulse. As shown in Table 1, the variants do not cease DNA synthesis after reaching their saturation density. However, the rate of incorporation of ³H-thymidine decreases markedly to a rate which for different variants is between 13 and 27.5% of that found in log-phase cells. It is unknown whether the cells which continue to synthesize DNA eventually divide and slough the daughter cells off into the medium. Presumably, this occurs since the number of cells in the monolayer does not increase.

Agglutinability. The con A-resistant SV3T3 cells were tested for their ability to be agglutinated by the lectins, concanavalin A and wheat germ agglutinin. As seen in Table 2, CA*32 and CA*41 did not agglutinate even in the presence of very high concentrations of lectins, and their behavior was indistinguishable from that of 3T3 cells. Although CA*30 was agglutinated by lower concentrations of the lectins, it required 32 times more con A and 25 times more wheat germ agglutinin than was needed to achieve half-maximal agglutination of the parental SV3T3 cells.

Mild trypsinization of the variants, a treatment which has been shown to render 3T3 cells highly susceptible to agglutination by con A or wheat germ agglutinin, caused the variants to become as agglutinable as untreated SV3T3 cells.

Resistance to the toxic effects of con A. The resistance of the variant clones of SV3T3 cells to the toxic effect of con A was determined by exposing the cells to various concentrations of the lectins as described in the legend to Fig. 4

and counting the number of clones of cells which grew after serial dilution of the treated cells. As seen in Fig. 4, all three of the variants were considerably more resistant to the toxic effects of the lectin than were the parental SV3T3 cells. At a dose of lectin ($300 \mu\text{g/ml}$) which was sufficient to kill over 99.99% of SV3T3 cells, about 1% of CA'32 and CA'41 cells survived. CA'30 cells were more sensitive to the lectin but still showed considerably greater resistance than did SV3T3 cells. From these

FIG. 2. Saturation density (2a) and ability to remain at that saturation density (2b,2c) of the cell line were determined by growth curves performed as described in text. (2a) Cells were seeded at a density of 10^4 cells/cm² and the medium was changed on the third and sixth days. (2b) Cells were seeded at a density of 10^4 cells/cm² and the medium was changed on the fourth day. (2c) Cells were seeded at a density of 5×10^4 cells/cm². The medium was either not changed (—) or was changed on the third and sixth days (-----). Symbols: SV3T3 (○), 3T3 (●), CA'30 (■), CA'32 (◐), CA'41 (▲).

FIG. 3. Ability of the con A-resistant cell line to grow in 1% serum, determined as described in text. Symbols: SV3T3 (O), 3T3 (●), CA'30 (■), CA'32 (•), CA'41 (▲).

experiments it seems that the CA'32 and CA'41 cells are as insensitive to the killing effect of con A as are 3T3 cells. CA'30 cells have a sensitivity which is intermediate between those shown by 3T3 and SV3T3 cells.

Binding of radioactive con A. The number of binding sites for ^{125}I -labeled con A on 3T3, SV3T3, and the variant cells was determined as described previously (25). The results are shown in Fig. 5, and it is apparent that there is little difference in the number of con A molecules bound by the three types of cells. In each case the total number of con A molecules bound per cell was about 1.2×10^7 —a figure which is in good agreement with earlier estimates (3, 25).

T antigen and rescue of SV40 virus. To determine whether the con A-resistant variants still contained functional virus genome(s), the cells were examined for the presence of SV40-specific T antigen by immunofluorescence. One hundred percent of the cells of each of the lines were positive, and there was no discernible difference between the intensity of fluorescence in the nuclei of the con A-resistant cells and the parental SV3T3 cells.

After 3T3 cells have been transformed by SV40, it is usually impossible to find any evidence of infectious virus, and attempts to induce virus replication by physical and chemical means have failed. However, if SV3T3 cells

TABLE 2. Agglutination by con A and wheat germ agglutinin (WGA)^a

Cell line	Con A ($\mu\text{g/ml}$ to give half-maximal agglutination)		WGA ($\mu\text{g/ml}$ to give half-maximal agglutination)	
	Not treated	Trypsin- ized	Not treated	Trypsin- ized
3T3	640	20	500	10
SV3T3	10	20	10	10
CA'30	320	20	250	10
CA'32	640	40	500	10
CA'41	640	20-40	500	10

^a Agglutinations were performed as described previously (25). Half-maximal agglutination equals 50 to 65% of the cells in clumps.

are fused with permissive monkey cells, infectious SV40 is synthesized in the heterokaryons. To test whether the changes in phenotype of the con A-resistant cells were a consequence of alterations in the resident virus genome(s), heterokaryons were formed between the variants and BS-C-1 cells as described in Materials and Methods. At the end of 10 days the heterokaryons were assayed for the presence of SV40. Table 3 shows that infectious SV40 was rescued from all of the lines of con A-resistant SV3T3 cells, although less efficiently than from the parental cells. The reason for this lower efficiency is unknown. The most likely explanation seems to be that the variants form heterokaryons with BS-C-1 cells less readily than do SV3T3 cells.

The virus rescued from the con A-resistant variants was passed twice on MA-134 cells in order to obtain high-titer stocks. The transforming ability of this virus was then assayed on 3T3 cells. As shown in Table 3, the virus rescued from the con A-resistant variants was as efficient at transforming as was virus rescued from SV3T3 cells. Thus, at least some of the SV40 rescued from the con A-resistant cells is not defective because it is both infectious and carries transforming ability.

Retransformation. Because revertants show essentially the same phenotype as untransformed cells, it is possible to determine whether they can be retransformed by infection with homologous or heterologous virus. The con A-resistant variants were exposed to SV40 and assayed for transformation as described in Materials and Methods. Table 4 shows that, whereas 3% of control 3T3 cells became transformed after infection with SV40, there was no detectable retransformation of the con A-resistant variants. The reason for this result is

unknown, but it is in agreement with experiments using other types of revertants, or lines of SV40-transformed cells with low saturation densities (29; also Vogel and Pollack, unpublished data). Recently, however, Renger (29) has retransformed temperature sensitive "revertants" of SV3T3 cells with murine sarcoma virus. When the con A-resistant SV3T3 cells were infected with a dose of MSV sufficient to transform 3T3 cells with very high efficiency, only occasional foci of transformants were observed (see Table 4), and a majority of the cells did not assume the morphology characteristic of MSV-transformed cells. It seems then that the con A-resistant variants successfully withstand all attempts to return them to a transformed state. There are many possible interpretations of this result, some of which are discussed later. One obvious possibility—that the con A-resistant cells do not absorb MSV—seems to be eliminated by the data in Table 4, where it is shown that cultures of the con A-resistant cells infected with MSV yield infectious virus. Under conditions where the number of MSV transformants of the con A-resistant clones was 1,000-fold lower than on control 3T3 cells, the titer of MSV produced was 1% of that obtained from the similarly infected 3T3 cells. Part of this

FIG. 4. Sensitivity of the various cell lines to the toxic effects of con A was determined as follows: 2×10^6 cells of each cell line were exposed to various concentrations of con A for a 24-h period. The dead cells were then washed off the plate. The remaining cells were detached from the plate with trypsin, counted, serially diluted, replated, and allowed to grow into clones. The clones were stained with hematoxylin and counted to determine the number of cells which survived the exposure to con A. Symbols: SV3T3 (○), 3T3 (●), CA'30 (■), CA'32 (◆), and CA'41 (▲).

FIG. 5. The number of con A molecules bound per cell was determined by the method of Ozanne and Sambrook (25) using ^{125}I -labeled con A with a specific activity of 1,500 counts per min per μg of con A. (●) ^{125}I -con A counts/min bound to cells in the absence of α -methylglucose (αMG). (▲) ^{125}I -con A counts/min bound to cells in the presence of αMG . (■) Difference in the counts/min of ^{125}I -con A bound to cells in the absence and presence of αMG . This represents the number of con A molecules specifically bound per cell.

difference in virus yield could be due to the fact that 3T3 cells infected with MSV multiply and the dividing cells all yield virus. The con A-resistant variants do not increase in number after exposure to MSV, so that the virus is produced only by cells present at the time of infection.

DISCUSSION

The work described here has utilized the selective toxicity of con A for transformed cells in order to isolate variants which are not killed by the lectin. The mechanism by which the cells have become resistant to the lectin is not known, but for two reasons it does not appear to involve a general loss of con A receptor sites. Firstly, mild trypsin treatment of the variants fully restores their capacity to be agglutinated by the lectin, and secondly, the cells bind as many con A molecules as do the original transformants. An alternative hypothesis is that the

TABLE 3. Virus rescue

Cell line	T-Antigen	Virus rescue ^a (PFU/10 ⁶ cells)	Transformation with rescued virus	
			PFU/ml ^b	% Transformants ^c
SV3T3	+	9.5×10^3	2.6×10^6	3
CA ³⁰	+	40	1.3×10^6	2.1
CA ³²	+	5	5.1×10^5	1.1
CA ⁴¹	+	2.75×10^3	1.7×10^6	3.2
3T3	—			

^a PFU/10⁶ cells of rescued virus from fusions of SV3T3 cells or the variants with BS-C-1 cells as described in Materials and Methods.

^b PFU/milliliter of lysate of rescued virus after having been passaged two times in MA-134 cells.

^c Percent of 3T3 cells transformed by rescued SV40 after two passages through MA-134 cells.

cells have lost a small number of con A sites which are specifically responsible for the sensitivity of the cells to the lectin. However, this

TABLE 4. *Retransformation*

Cell line	SV40 ^a	MSV ^b	FFU/ml of media ^c
3T3	3.1%	3×10^4	5×10^5
CA*30	0	4	2.1×10^5
CA*32	0	7	5×10^5
CA*41	0	5	3×10^5

^a Percent transformation of 3T3 and variants after infection by SV40 stock virus with a titer of 5×10^6 PFU/ml.

^b Focus forming units (FFU)/milliliter of MSV stock solution titered on 3T3 and the variants.

^c FFU/milliliter of MSV virus on 3T3 cells. The virus stocks were obtained from 3T3 cells or the variants 10 days after infection with 3×10^4 FFU/ml of MSV.

explanation too, is unsatisfactory because the cells resistant to con A are not agglutinated by the unrelated lectin, wheat germ agglutinin. Concanavalin A and wheat germ agglutinin do not compete for the same binding sites on cells (25), and separate receptors for each lectin have been isolated (V. K. Jansons and M. M. Burger, *Fed. Proc.*, p. 692, 1971), so that it is impossible to explain the phenotype of the con A-resistant variants solely in terms of a loss of a small number of con A receptor sites. Perhaps the most reasonable explanation is that the surface of the variant cells has undergone a more general change so that the con A and wheat germ receptor sites, rather than remaining in the clumped configuration typical of transformed cells, have returned to the disperse topology found in 3T3 cells (22). However, we cannot exclude other possibilities. For instance, the increased synthesis of sialic acid which other workers have reported to occur in con A-resistant cells (8) may provide a layer on the surface which somehow shields the cell from the effects of the lectin.

Whatever the nature of the change, it affects not only the way the cells interact with con A and wheat germ agglutinin, but also their social behavior. Unlike SV3T3 cells, the con A-resistant variants will not form multilayers in culture. Once a confluent sheet of cells has formed, the rate of cell division is markedly reduced. By contrast to 3T3 cells, however, DNA synthesis continues, albeit at a reduced level. It seems then that the con A variants do not display a complete return to a normal phenotype, but rather that they represent an intermediate stage between full-blown transformants and normal cells.

As is shown in the following paper, the con A-resistant cells contain viral DNA (26), which

is transcribed in a pattern indistinguishable from that found in the parental SV3T3 cells. T antigen is present in the nuclei, and infectious virus from the variants can be rescued by heterokaryon formation. The simplest conclusion from these data is that the phenotypic change exhibited by the con A-resistant cells is due not to an alteration in the virus genome, but to a variation in the cell. Unfortunately, this argument alone is not conclusive. We know the variant cells contain multiple copies of the SV40 genome, and there is no guarantee that the copy of the virus rescued was the one maintaining the cells in a transformed state. The possibility that con A-resistant variants are in fact deficient in a virus function and do not display the full panoply of transformation for this reason cannot be excluded. However, the failure of the variants to be transformed by a heterologous virus, MSV, strongly suggests that an altered cellular function is responsible for the revertant phenotype.

In the case of SV40 we have no evidence to disprove the possibility that the cells are altered so the superinfecting virus is blocked during adsorption, penetration, or uncoating. However, this cannot account for the failure of the cells to become transformed by MSV. Undoubtedly, MSV infects the cells because infectious virus is produced. This result implies that there are some common pathways used both by MSV and by SV40 to cause transformation and suggests that the con A-resistant variants may be blocked in one of these pathways. The nature of this putative block is totally obscure. A simple-minded suggestion is that the event responsible for the failure of the con A-resistant variants to retransform also accounts for the atavistic behavior of these cells. After all, the con A variants have been able to reverse some of the effects of the original transforming event, so perhaps it is not surprising they can surmount the challenge of a second transforming virus.

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ADDENDUM

After this manuscript was submitted, a report (K. D. Noonan, H. C. Renger, C. Basilio, and M. M. Burger, 1973. Surface changes in temperature-sensitive simian virus 40-transformed cells. *Proc. Nat. Acad. Sci. U.S.A.* 70:347-349) appeared which demonstrated that SV3T3 cells bound four to five times

the amount of con A that bound to 3T3 cells. Since the con A binding experiments reported by Noonan et al. were performed under different assay conditions than those used in this paper, it is difficult to compare the two sets of results.

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