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In Vitro Transformation of Rat and Mouse Cells by DNA from Simian Virus 40

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Primary rat kidney cells and mouse 3T3 cells can be transformed by DNA of simian virus 40 when use is made of the calcium technique (Graham and van der Eb, 1973). The transformation assay in primary rat cells is reproducible, but the dose response is not linear.

The purified DNA of simian virus 40 (SV40) is biologically active and capable of causing a productive infection in permissive cells. Several methods have been used to demonstrate infectivity of viral DNA, the most efficient and commonly used method being the DEAE-dextran technique (6, 9, 13). Although high specific infectivities of greater than 10^6 PFU/μg of DNA can be obtained with the latter technique, it does not appear to be suitable for inducing transformation of nonpermissive cells (10; P. J. Abrahams, unpublished observation). Black and Rowe (3) described an apparent transformation of BHK-21 cells with SV40 DNA using the hypertonic saline method. However, it was uncertain whether the observed transformation was SV40 specific. Furthermore, successful transformation of human cells by SV40 DNA has been reported using the DEAE-dextran technique (1, 2). Human cells, however, are semipermissive for SV40, and it is possible that in this case transformation was caused by intact virus which had been produced by a preceding lytic response rather than by the viral DNA.

We have recently demonstrated that primary rat kidney cells can be transformed by DNA of adenovirus types 2, 5 and 12 (5, 7). These results were obtained with the calcium technique, which was originally developed for assaying infectivity of adenovirus DNA (6). In the present paper we describe the successful transformation of primary rat kidney cells and mouse 3T3 cells with SV40 DNA by means of the calcium technique.

SV40 clone 307 L from S. Kit, was grown in BSC-1 cells infected at a multiplicity of 10^{-3} PFU/cell. Viral DNA was extracted from infected cells using the Hirt procedure (8) and was purified by ethanol precipitation and two or three cycles of banding in CsCl equilibrium gradients (density, 1.56 g/cm^3) containing 200 μg of ethidium bromide per ml.

Only the closed-circular DNA (form I) present in the denser band was used. Routinely, the DNA was labeled with [^{3}H]thymidine (1.5×10^3 to 3.5×10^3 counts per min/μg of DNA). Transformation assays were carried out with primary cultures of rat kidney cells as described previously (6, 7), except that the DNA-calcium phosphate precipitate was added to the cultures without removing the medium. Briefly, primary cultures of kidney cells from 6- to 7-day-old Wistar rats were made in 5-cm petri dishes. One day prior to infection with DNA, the medium of the cultures was changed with 5 ml of fresh medium (Eagle minimum essential medium plus 10% calf serum).

At the time of the infection, the monolayers were 70 to 80% confluent. The cells were infected by adding 0.5 ml of a mixture containing viral DNA (0.02 to 8.00 μg), 5.0 μg of salmon sperm DNA, and 125 mM $CaCl_2$ in HEPES-buffered saline, pH 7.05, per dish.

After 3 to 4 h of incubation at 37 C the medium was replaced by fresh medium, and the incubation was continued for 3 weeks. During this period the medium was changed twice weekly. Foci of highly packed, multilayered cells appeared as early as 2 weeks after infection, and were counted at 3 weeks, after the cells had been fixed and stained with Giemsa. Control dishes, which were mock infected with salmon sperm DNA only or received no treatment at all, were always included in each experiment but were never found to contain such transformed foci.

Several colonies were isolated and established as cell lines, and four lines were tested for the presence of SV40-specific T antigen by the indirect immunofluorescence method, using serum from tumor-bearing hamsters and fluorescein isothiocyanate-conjugated swine anti-hamster serum (11). All four lines were found to contain SV40-specific T antigen in 95 to

100% of the cells. In one line, the intensity of fluorescence was very low but was still clearly positive compared to primary untransformed kidney cells. To ensure that the observed transformation was actually due to the viral DNA, and not to intact virus which was produced by a lytic reaction in the rat kidney cells, the following experiment was carried out. Primary rat kidney cells were infected with 1 μg of SV40 DNA per 5-cm dish, and 24, 48, 72, and thawed and tested for the presence of infectious virus on BSC-1 cells. No infectious virus could be detected in any of the cultures. To test the possibility that SV40 DNA replication occurs in DNA-infected rat kidney cells, cultures were infected with 1 μg of SV40 DNA per dish, and 24 and 48 h after infection the cultures were pulse labeled with [^{3}H]TdR (10 μCi/ml of medium) for 2 h. DNA was extracted from the cultures by the procedure of Hirt (8), and the Hirt supernatant was analyzed by velocity sedimentation in neutral 5 to 20% sucrose gradients (SW41 Ti Spinco Rotor, 40,000 rpm, 5½ h). ^{14}C-labeled SV40 DNA was added as a marker. No ^{3}H-labeled DNA was found to sediment in the area of the marker DNA, indicating that no detectable viral DNA was synthesized.

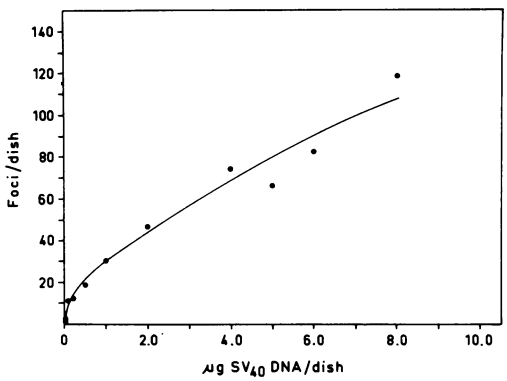

FIG. 1. *Dose response for transformation of primary rat kidney cells by form I SV40 DNA. The points represent the total average number of foci per dish (see Table 1).*

Figure 1 shows the dose response for transformation of primary rat kidney cells with form I SV40 DNA. As can be seen the curve is not linear, in contrast to the dose response obtained for transformation with adenovirus 5 DNA (5). The transformation efficiency for SV40 DNA appears to be highest at low viral DNA concentrations, decreases with increasing concentration, and levels off to an almost constant value above 1 μg per dish. This is more clearly demonstrated in Table 1, in which the calculated average specific transforming activities are shown. For DNA concentrations higher than 1 μg per dish, values of 14 to 23 foci per μg of DNA were found, whereas considerably higher numbers were noted below 1 μg per dish.

The deviation from linearity cannot be caused by incorrect scoring of the colonies due to overlapping, since the effect is most clearly seen in the range below 2 μg of DNA per culture, where the numbers of transformed foci per dish are still low. No explanation has been found for this phenomenon. A rather similar dose-response relationship has been reported for transformation of BHK-21 cells by polyoma virus DNA (4). In the latter study, a decrease in specific transforming activity was likewise observed with doses higher than 1 μg of DNA per inoculated culture.

Table 1 also shows that, although small

TABLE 1. *Transformation of primary rat kidney cells by form I SV40 DNA*

DNA/dish (μg)	Avg no. of foci/dish[a]				Total avg no. of foci/dish[b]	Total avg no. of foci/μg of DNA
	Expt 1	Expt 2	Expt 3	Expt 4		
0.02			1.0+		1.0	50.0
0.05				2.7 ± 1.3	2.7	54.0
0.1	7.0 ± 4.2	9.5 ± 2.1	15.0+	13.0 ± 4.2	11.1	111.0
0.2			12.0+		12.0	60.0
0.5	14.3 ± 3.5			23.3 ± 7.5	18.8	37.6
1.0	25.3 ± 3.7	28.0 ± 2.8	43.0 ± 10.1	23.6 ± 8.9	30.0	30.0
2.0	41.0 ± 1.7		65.0 ± 7.1	33.3 ± 10.0	46.3	23.2
4.0	78.0 ± 6.1		78.0+	68.3 ± 5.7	74.7	18.6
5.0		66.5 ± 3.5			66.5	13.3
6.0			89.3 ± 7.6	76.6 ± 9.3	82.9	13.8
8.0	119.0 ± 0				119.0	14.9

[a] Most values represent the average of two to three dishes, ± the standard deviation, except the cases marked by +, where one dish per DNA concentration was used.

[b] Averages of up to four different experiments. These values are presented graphically in Fig. 1.

variations in transforming efficiency occur in different experiments, the dose response is rather reproducible. It has also been possible to transform an established line of mouse cells with SV40 DNA. A clone of 3T3 cells, isolated from a BALB/c 3T3 line obtained from G. Todaro, was used for this experiment. The cells were grown and maintained in Dulbecco modified Eagle medium supplemented with 10% calf serum (12). Monolayers in 5-cm petri dishes (Falcon) were infected with 0.5 to 4.0 μg of SV40 DNA per dish when the cultures were 60 to 70% confluent. After 1 day, the cultures were trypsinized and the cells were counted and replated at 10^2, 10^3 and 10^4 cells per dish. After 5 to 6 weeks, transformed foci were observed in the cell cultures which were originally infected with the highest DNA concentration (Table 2). Three clones were grown and tested for the presence of SV40-specific T antigen by indirect immunofluorescence (11). All three lines were found to contain SV40-specific T antigen in 95 to 100% of the cells. When the transforming activity was expressed on the basis of 35 foci per 10^3 cells, a specific activity of 2,600 foci per μg of DNA was calculated, assuming a plating efficiency of 100%. The specific transforming activity of SV40 DNA on 3T3 cells might therefore be considerably higher than 2,600 foci/μg of DNA, depending on the actual plating efficiency. When the experiment was completed after 7 weeks, a confluent background of untransformed cells was present in each dish, so that the actual plating efficiency could not be determined in this experiment. In spite of the high efficiency of transformation obtained with 3T3 cells, we found it more convenient to use primary rat kidney cells for the assay, because in our hands 3T3 cells were less easy to handle and to maintain in a sufficiently contact-inhibited condition suitable for transformation (12).

TABLE 2. *Transformation of BALB/c 3T3 cells by SV40 DNA*[a]

No. of cells plated/dish	Foci/dish	
	DNA infected	Uninfected[b]
10^2	2	2
10^3	35	2
10^4	23	2

[a] 3T3 cells infected with 4 μg of SV40 DNA per 5-cm dish were replated in different concentrations, 1 day after infection, using one dish per concentration. Mock-infected control cultures were similarly treated.

[b] The foci observed in the uninfected control cultures were much smaller (1 to 2 mm) and less dense then the foci in the DNA-infected cultures at the same age (diameter 4 to 6 mm).

In conclusion, we have demonstrated that it is possible to obtain a reproducible transformation of cells in vitro with SV40 DNA when use is made of the calcium technique. It was recently reported that transformation has also been obtained with DNA from polyoma virus (M. Fried, personal communication) and with herpes simplex DNA(N. M. Wilkie, J. B. Clements, J. C. M. Macnab, and J. H. Subak-Sharpe, Abstr. Meet. Cold Spring Harbor Symp. Quant. Biol., 1974, **39**:32) using the calcium technique. It is surprising that the DEAE-dextran technique gave negative results for transformation, whereas it turned out to be more efficient for the assay of SV40 DNA infectivity than the calcium technique. With the calcium technique, an infectivity of approximately 10^4 PFU/μg of DNA was found (6), in contrast to 10^5 to 10^6 PFU/μg of DNA with the DEAE-dextran technique (P. J. Abrahams, unpublished data). We have no explanation for the failure to obtain transformation by SV40 DNA with the DEAE-dextran technique. Preliminary experiments have shown that it is also possible to transform rat kidney cells with linear SV40 molecules and with specific DNA fragments, suggesting that it might be possible to isolate the segment of the DNA which is responsible for transformation (5).

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