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New Procedure for DNA Transfection with Polycation and Dimethyl Sulfoxide

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A new procedure for DNA transfection has been developed in a system of chicken embryo fibroblast cells and cloned Rous sarcoma virus DNA by using a polycation reagent as a mediator to adsorb DNA to the cell surface and dimethyl sulfoxide as an agent to facilitate the uptake of adsorbed DNA by the cells. In this new, simple, and convenient polycation-dimethyl sulfoxide transfection, which requires no carrier DNA even with small amounts of DNA, the number of transformed cell foci induced by Rous sarcoma virus DNA was proportional to the dose of the transfecting DNA, and chicken embryo fibroblast cells were successfully transformed by v-*src*-containing subgenomic DNA as well.

The transfection technique has been widely used to investigate the biological activities of cellular and viral DNAs directly extracted from various tissues, cells, and virions or those molecularly cloned in bacteria. This technique has contributed greatly to the recent rapid progress in studies on oncogenes in human cancers (1, 21). Of the various methods developed for transfection of tissue-cultured cells (11, 14, 19, 20), the calcium phosphate method has been employed most often because it is more efficient and reproducible than other methods (5). However, the calcium phosphate method has narrow optimal conditions for several factors, such as the pH of the buffer used, the amount of the DNAs, and the size of the calcium phosphate-DNA precipitate. Thus, the efficiency of transfection depends greatly on the conditions used (5, 8, 10). Furthermore, the mechanism for DNA uptake by the cell from calcium phosphate-DNA precipitate has not been fully elucidated (10). We attempted to develop a simpler and easier procedure for DNA transfection by directly adsorbing DNA to the cell surface instead of forming a calcium phosphate-DNA coprecipitate.

Transfection seems to involve two steps, adsorption of DNA to the cell surface and then DNA uptake by cells. Polycations are known to enhance adsorption of retroviruses to cells (17, 18), probably by interacting with negative charges of both virions and cell surfaces and thus forming bridges between the two. Therefore, DNAs would likewise be adsorbed to the cell surface in the presence of polycations since DNA molecules have a polyanionic character. In calcium phosphate transfection, brief treatment of cells with 25 to 30% dimethyl sulfoxide (DMSO) appears to increase the transfection efficiency (8, 9, 16). DMSO is considered to enhance uptake of adsorbed DNAs by increasing the permeability of cell membranes. Therefore, we first examined the effects of the combination of the polycation Polybrene, which facilitates retrovirus adsorption (17), and DMSO on DNA transfection. As shown below, the Polybrene-DMSO combination was found to be very effective.

In this study, the transfecting agent used was a molecularly cloned Rous sarcoma virus (RSV) DNA derived from plasmid pSRA-2, which was prepared by DeLorbe et al. (3). This plasmid DNA contains the entire genomic sequence of RSV virus in a permuted form. Before transfection, pSRA-2 DNA was digested with *Sal*I and religated. Transfection efficiency was determined by measuring focus formation on chicken embryo fibroblast (CEF) cells. Focus formation in RSV DNA-transfected CEF cell cultures depends on secondary infection with virus released from transfected cells (2). Therefore, the amount of effective RSV DNA was calculated by assuming that one-fourth of the ligated viral DNA was in the right configuration for expressing viral genes and producing infectious virus.

Inocula of 8×10^5 CEF cells were seeded in 60-mm dishes; 18 h later the culture fluid was removed, and 50 ng of RSV DNA in 1 ml of fresh medium was added with various amounts of Polybrene (Aldrich Chemical Co., Milwaukee, Wis.). The cultures were incubated for 6 h with occasional shaking to enhance adsorption. The culture fluid was then removed, and the culture was treated with 2 ml of medium containing 20 to 30% DMSO at room temperature for 4 min. The culture was washed once with medium and incubated with 2 ml of fresh medium at 37°C for 20 to 24 h. Then, it was overlaid with medium containing agar. Transformed foci were scored on day 7 after transfection. Under the optimum conditions, 50 ng of RSV DNA induced about 1,000 foci (Table 1). Although the transfection efficiency by the calcium phosphate method varies from experiment to experiment, the same amount of RSV DNA induced, at most, 100 foci in our experiments. However, a precise comparison of the efficiency of transfection by this method with that by the calcium phosphate method was not possible because we were not certain whether we employed the optimal conditions in transfection required by the calcium phosphate method. The efficiency of transfection depends on the concentration of DMSO used. The maximum efficiency was usually obtained with cultures which showed light cytopathic effect after DMSO treatment. Therefore, for the maximum efficiency of transfection, there seems to be a critical concentration at which increase in cell permeability is balanced by decrease in cell viability. In the case of CEF cells, the optimal concentration of DMSO appears to be ca. 30%. Concentrations of more than 30% DMSO and more than 30 μg of Polybrene per plate had marked cytopathic effects. The efficiency of transfection also depends on the order of DNA addition and DMSO treatment. When cells were treated with DMSO first and then DNA and Polybrene were added, there was no detectable transfection. Washing the 30% DMSO-treated cultures with 15% DMSO before addi-

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TABLE 1. Effects of Polybrene and DMSO on transfection

Expt	Concn of DMSO (% [vol/vol])	No. of foci per plate with Polybrene treatment (μg per plate)[a]				
		30	10	3	1	0
1	30.0	1,000	574	352	264	4
	27.5	1,024	467	467	148	2
	25.0	232	241	188	44	2
	20.0	72	84	42	15	2
	0	0	0	0	0	0
2	30.0	1,168	688	472	196	8
	27.5	408	NT[b]	420	NT	32
	25.0	348	NT	248	NT	24
	20.0	184	NT	108	NT	12
	0	52	NT	40	NT	0

[a] A stock solution of Polybrene (10 mg/ml) was diluted with phosphate-buffered saline before use. Samples (0.1 ml) of appropriate dilutions were added to plates which contained 1 ml of medium supplemented with 50 ng of RSV DNA.
[b] NT, Not tested.

tion of medium reduced cytopathic effect considerably and, at the same time, diminished transfection. These facts suggested that osmotic shock, in addition to the change in cell permeability by DMSO treatment, plays an important role in the DNA uptake.

Incubation of CEF cells with DNA in the presence of Polybrene (30 μg per plate) for various periods showed that the transfection efficiency increased for up to 6 h (Fig. 1) and then gradually decreased. Therefore, adsorption of DNA onto cell surfaces by virtue of Polybrene proceeds rather slowly. When CEF cells were seeded with Polybrene 18 h before DNA addition, DNA was found to be adsorbed to cells much faster, and the maximum transfection was attained by 3 h of incubation with DNA.

Under conditions in which the concentrations of both Polybrene and DMSO appeared to be optimal, focus formation by RSV DNA was proportional to the DNA dose (Fig. 2), indicating that no carrier DNA is required for transfection in this system even with small amounts of DNA. Cultures transfected with more than 50 ng of DNA contained too many foci to count, but linearity of the response appeared to be maintained with doses of up to several hundred ng of DNA, which apparently induced full transformation of cultures.

Recently, Fung et al. (4) developed procedures employing direct injection of DNAs into chickens to test the transforming activity of cloned subgenomic *src* DNA fragments. Such testing is difficult in the in vitro transfection system with the calcium phosphate method. To test the validity of the new polycation-DMSO procedure, we also tried to transfect CEF cells with subgenomic *src* DNA as described above. The *Eco*RI-B fragment derived from pSRA-2 DNA, which contains the entire *src*-coding sequence, a portion of the long-terminal-repeat sequence, and about 700 nucleotides of the *env* sequence, has been subcloned in the recombinant plasmid, psrc#2 (3). The subgenomic *src* DNA was generated by digestion of the psrc#2 DNA with *Eco*RI. CEF cells were transfected with the subgenomic DNA at 1 μg of *src* DNA per plate. A total of 21 foci appeared on two transfected plates within 6 to 8 days. The foci consisted of round cells with the morphological characteristics of RSV-transformed cells. No infectious virus was demonstrated in these cultures. The dose response of colony formation of transformed cells was examined in this system. Higher DNA input gave more transformed cell colonies, although the relative efficiency of transfection was reduced with higher input of DNA (Table 2). These results suggested that the efficiency of transfection by the new procedure with Polybrene and DMSO was comparable to that by the in vivo system described by Fung et al. (4) and higher than that by the calcium phosphate method.

DEAE-dextran, another polycation that is also used for retrovirus adsorption, has been used as a facilitator in transfection at relatively high concentrations (100 to 1,000 μg/ml) (11, 14, 19); however, the mechanism of DNA uptake by cells in the presence of this reagent remains obscure. We found that it also worked as an efficient mediator to adsorb DNA to cells at a narrow range of effective concentrations (1 to 10 μg/ml). However, DEAE-dextran did not induce detectable transfection at this range of concentrations without DMSO treatment. DEAE-dextran facilitated DNA adsorption to cells much faster than Polybrene (1-h incubation of cultures with DNA and DEAE-dextran was enough to cause maximum transfection). This efficient adsorption by DEAE-dextran is probably due to the difference in the

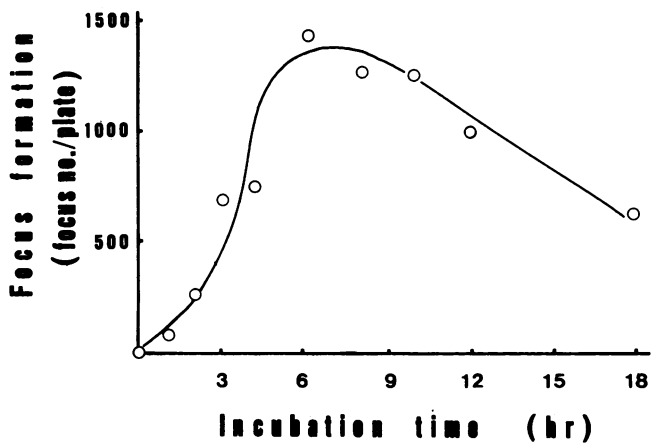

FIG. 1. Time course of DNA adsorption to cells. CEF cells were incubated with 1 ml of medium containing 50 ng of RSV DNA and 30 μg of Polybrene for various times and then treated with 30% DMSO as described in the text.

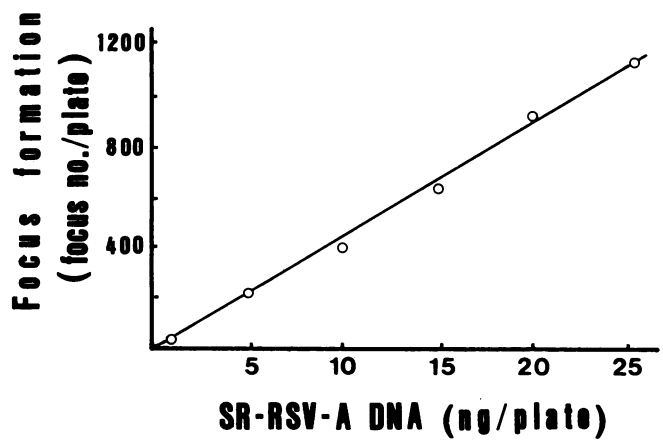

FIG. 2. Dose response of transfection. CEF cells were incubated with various amounts of RSV DNA in the presence of 30 μg of Polybrene for 6 h and then treated with 30% DMSO.

TABLE 2. Colony formation by v-*src* DNA[a]

Amt of v-*src* DNA (ng per plate)	No. of colonies formed[b]	
	Expt 1	Expt 2
0	0, 0	0, 0
100	1, 3	2, 3
300	3, 6	7, 4
1,000	12, 13	12, 16
3,000	18, 20	27, 29

[a] CEF cells were seeded with Polybrene (10 μg per plate), and 18 h later the cells were incubated with various amounts of *Eco*RI-digested psrc#2 DNA (v-*src* DNA) for 3 h and then treated with 30% DMSO. At 18 h after DMSO treatment, the transfected cells were suspended in 10 ml of soft agar-containing medium as described elsewhere (7) and incubated for 2 weeks.

[b] Results are for two plates in each experiment.

molecular size of the two reagents (the average molecular weights of Polybrene and DEAE-dextran are 6,000 and 10^6, respectively). We also tested UV-inactivated Sendai virus and polyethylene glycol as substitutes for DMSO because both of these reagents are known to cause membrane alteration resulting in cell-to-cell or cell-to-virus fusion (6, 12, 13, 15). However, these reagents were significantly less effective than DMSO.

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