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Short-Term, High-Efficiency Expression of Transfected DNA

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We have achieved high-efficiency uptake and expression of foreign DNA in mouse Ltk$^-$ cells by modifying the DEAE-dextran-mediated transfection method of McCutchan and Pagano (J. Natl. Cancer Inst. 42:351–357, 1968) to include an initial incubation at elevated pH followed by a shock treatment with dimethyl sulfoxide. Up to 80% of mouse Ltk$^-$ cells transfected with the herpes simplex virus thymidine kinase gene expressed thymidine kinase as measured by autoradiography.

The short-term expression of DNA transfected into eucaryotic cells enables the study of the function of the DNA (e.g., RNA transcripts and protein-coding capability) shortly after transfection. The time required for long-term selection and the possibility of DNA rearrangements are avoided. The success of short-term-expression studies depends on the efficiency of transfection, i.e., the fraction of cells expressing transfected DNA. By modifying the DEAE-dextran transfection method of McCutchan and Pagano (6) to include an intial incubation at elevated pH followed by a dimethyl sulfoxide (DMSO) shock, we have obtained expression of a transfected herpes simplex virus type 1 thymidine kinase (TK) gene in over 80% of mouse Ltk$^-$ cells (Fig. 1). The high-efficiency, short-term expression resulting from DEAE-dextran-mediated DNA transfection did not produce stable transformants, i.e., no colonies were obtained from transfected Ltk$^-$ cells in HAT-selective media (containing hypoxanthine, aminopterin, and thymidine).

The observation of Zetterberg and Engstrom (14) that pH elevation has a mitogenic effect on cells led us to ask whether pH elevation would increase DNA transfection efficiency. Incubation at 2% CO_2 raised the pH of the incubation mixture, as indicated by the color of the neutral red indicator in the media. When we used the DEAE-dextran transfection method of Sompayrac and Danna (11), less than 1% of Ltk$^-$ cells expressed the transfected herpes simplex virus TK gene. A 1-h incubation at 2% CO_2 during exposure to DEAE-dextran increased the number of expressing cells from 1% to between 2 and 5%. When combined with the DMSO shock treatment described below, the optimum time of incubation at 2% CO_2 was 1 h, which doubles the number of cells expressing the TK gene (Table 1). Longer incubation at low CO_2 concentration decreased cell survival and reduced the number of TK-positive cells obtained.

Maximal transfection efficiency occurred after 4 h of incubation in DEAE-dextran (Fig. 2). The number of cells expressing the TK gene started to decline when cells were subjected to DEAE-dextran incubations of more than 6 h. Long exposure to DEAE-dextran also resulted in a decrease in cell survival. The optimum DEAE-dextran concentration for transfection of the Ltk$^-$ cells was 500 μg/ml (Table 1). The number of TK-positive cells decreased dramatically at lower and higher concentrations of DEAE-dextran. The concentration of DEAE-dextran and the time of exposure may have to be adjusted to match the tolerance of different cell lines. The source and molecular weight of the DEAE-dextran may be important. We obtained 15 to 20% fewer TK-positive cells when DEAE-dextran from Sigma Chemical Co. (molecular weight, 500,000) was substituted for that from Pharmacia Fine Chemicals, Inc. (molecular weight, 1,500,000).

The DMSO stimulation of stable transformant formation after chromosome-mediated gene transfer (7) and calcium phosphate-mediated transfection (5, 12) encouraged us to determine the effect of DMSO on DEAE-dextran transfection. The inclusion of a DMSO shock after DEAE-dextran exposure greatly increased the number of cells expressing the TK gene (Fig. 3). As illustrated, a 2-min DMSO shock increased the number of TK-positive cells from 6 to 73%. The optimum time of DMSO treatment was 2 min, after which the transfection efficiency began to decrease. Glycerol could partially substitute for DMSO. When 15% glycerol in HEPES-buffered saline was used in place of DMSO, 30 to 40% of the cells expressed the TK gene.

In microinjection experiments, Yamaizumi et al. (13) obtained a greater number of TK-positive cells than the number that they injected and attributed this to cross feeding between neighboring cells. To determine whether the DMSO treatment increased cross feeding rather than transfection efficiency, Ltk$^-$ cells were transfected as described in Fig. 1. Cells were harvested 4 h after the DMSO shock by using trypsin and were reseeded at 1/20 of their initial cell density, such that there was no apparent contact between neighboring cells. The low-density cells exhibited 70 to 80% of the number of TK-positive cells in the undiluted control cells, implying that DMSO treatment stimulates DNA transfection rather than cross feeding.

The effect of the plasmid DNA concentration in the transfection mixture is illustrated in Table 1. The optimum concentration of pGM6 DNA was 2 μg/ml. At a pGM6 concentration of 10 μg/ml, the number of expressing cells was reduced by one-half. At a pGM6 concentration of 0.1 μg/ml, less than 2% of the cells expressed TK. The addition of 2 μg of carrier DNA (sheared human placental DNA; Sigma Chemical Co.) per ml to 0.1 μg of pGM6 per ml did not increase the number of expressing cells. This suggests that the concentration of the TK gene in the transfection mixture and not the total mass of DNA determines the transfection efficiency. Reconstruction experiments suggest that the TK gene would not be detectable after transfection with genomic DNA. Experimentally, no TK-positive cells were detected when Ltk$^-$ cells were transfected with 2 μg of human placental DNA per ml.

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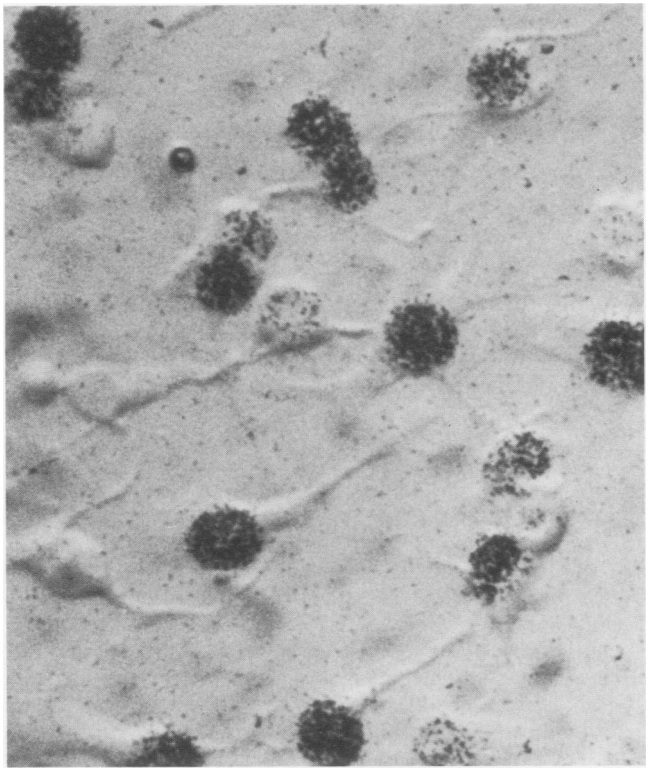

FIG. 1. Autoradiographic detection of TK after DNA transfection. The day before transfection, mouse Ltk$^-$ cells were seeded at 1 $\times$ 10^5 to 2 $\times$ 10^5 cells per 35-mm dish. The medium was aspirated, and each dish was rinsed with serum-free Dulbecco modified Eagle medium (DME). The transfection solution (0.7 ml per dish), consisting of 500 μg of DEAE-dextran per ml and 2 μg of pGM6 DNA per ml in serum-free DME, was added to the cells. Plasmid pGM6 (8) is a derivative of pBR322 containing the herpes simplex virus type 1 3.6-kilobase *Bam*HI restriction fragment coding for TK and the polyoma 3.6-kilobase *Bam*HI to *Hin*cII (58.0 to 26.5 map units) restriction fragment coding for the early functions of the virus. The cells were incubated at 37°C in the transfection solution for 1 h in 2% CO_2 and then for 3 h in 5% CO_2. The solution was removed, and 0.5 ml of 10% DMSO in HEPES-buffered saline (9) was applied. The cells were incubated in DMSO solution for 2 min at 37°C in 5% CO_2. The cells were rinsed with phosphate-buffered saline, and 2 ml of DME containing 10% fetal calf serum was added. The next day the medium was replaced with DME containing 10% fetal calf serum and 10 μCi of [^{3}H]thymidine (ICN, 80 Ci/mmol) per ml. The cells were incubated for 24 to 36 h, after which they were fixed and processed for autoradiography as previously described (8). The emulsion was exposed for 2 to 3 days before developing. The cells expressing the TK gene were easily identified by the presence of grains over their nuclei.

TABLE 1. Transfection parameters[a]

Parameter	TK-positive cells (%)
Incubation time at 2% CO_2 (h)	
0	31
0.5	49
1.0	59
1.5	53
2.0	36
3.0	32
DEAE-dextran concn (μg/ml)	
0	0
100	31
300	45
500	83
700	63
900	42
pGM6 DNA concn (μg/ml)	
0	0
0.01	0
0.1	1
1.0	58
2.0	77
3.0	70
4.0	56
5.0	52
10.0	39

[a] Exposure time of cells to DEAE-dextran was maintained at 4 h by adjusting the incubation time at 5% CO_2. Conditions for these experiments were the same as those described in the legend to Fig. 1, except for the indicated variable.

The high-efficiency DNA transfection method described above is effective both in other cell lines and for other genes. M. Hauser and P. Gearhart (unpublished data) have observed expression of TK in up to 96% of the TK$^-$ SP2/0 B-cell hybridoma (10) after transfection with pGM6 DNA. M. A. Lopata, D. W. Cleveland, and B. Sollner-Webb (unpublished data) have found a 50-fold increase in chloramphenicol acetyltransferase activity when a DMSO shock treatment is included after DEAE-dextran-mediated transfection of mouse L-cells with pSV2CAT DNA (3). In addition, they reproducibly obtained 5- to 10-fold higher levels of expression by the DEAE-dextran-DMSO treatment compared with calcium phosphate-mediated DNA transfection.

Other investigators have reported high DNA transfection efficiencies. Chu and Sharp (1) showed that 15% of CV-1 cells expressed transfected DNA by a modification of the DNA-calcium phosphate method described by Graham and van der Eb (4). Danna and Sompayrac (2) described expression in 50% of transfected BSC cells after 24 h of exposure to

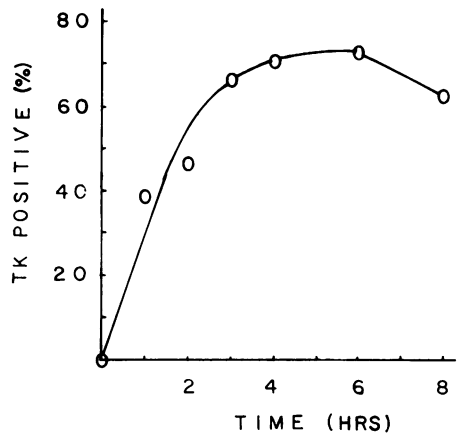

FIG. 2. Time of exposure to DEAE-dextran. The transfection was performed as described in the legend to Fig. 1, except that the time of exposure to DEAE-dextran at 5% CO_2 was varied. The times include the 1 h of initial incubation at 2% CO_2.

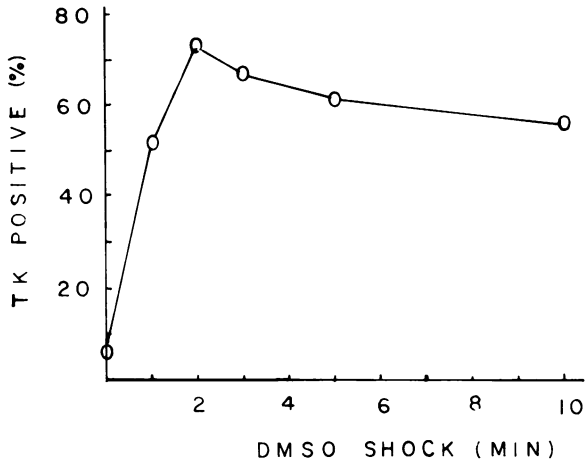

FIG. 3. DMSO enhancement of DNA transfection. The DEAE-dextran transfection was carried out as described in the legend to Fig. 1, except that the time of DMSO treatment was varied. A representative experiment is displayed. For each time point, the percentage of cells expressing the TK gene was determined by counting the number of TK-positive cells out of a total of 100 to 200 cells.

high concentrations of DEAE-dextran. The transfection procedure described in this report gives the highest efficiency reported.

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LITERATURE CITED

1. **Chu, G., and P. A. Sharp.** 1981. SV40 DNA transfection of cells in suspension: analysis of the efficiency of tanscription and translation of T-antigen. Gene **13**:197–202.
2. **Danna, K. J., and L. M. Sompayrac.** 1982. Efficient infection of monkey cells with SV40 DNA. II. Use of low-molecular-weight DEAE-dextran for large-scale experiments. J. Virol. Methods **5**:335–341.
3. **Gorman, C., G. Merlino, M. Willinghajm, I. Pastan, and B. Howard.** 1982. The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. Proc. Natl. Acad. Sci. U.S.A. **79**:6777–6781.
4. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
5. **Lewis, W. H., P. R. Srinivansan, N. Stokoe, and L. Siminovitch.** 1980. Parameters governing the transfer of the genes for thymidine kinase and dihydrofolate reductase into mouse cells using metaphase chromosomes or DNA. Somatic Cell Genet. **6**:333–347.
6. **McCutchan, J. H., and J. Pagano.** 1968. Enhancement of the infectivity of simian virus 40 deoxyribonucleic acid with diethylaminoethyl-dextran. J Natl. Cancer Inst. **41**:351–357.
7. **Miller, C. L., and F. H. Ruddle.** 1978. Co-transfer of human X-linked markers into murine somatic cells via isolated metaphase chromosomes. Proc. Natl. Acad. Sci. U.S.A. **75**:3346–3355.
8. **Milman, G., and M. Herzberg.** 1981. Efficient DNA transfection and rapid assay for thymidine kinase activity and viral antigenic determinants. Somatic Cell Genet. **7**:161–170.
9. **Parker, B. A., and G. R. Stark.** 1979. Regulation of simian virus 40 transcription: sensitive analysis of the RNA species present early in infections by virus or viral DNA. J. Virol. **31**:360–369.
10. **Shulman, M., C. D. Wilde, and G. Kohler.** 1978. A better cell line for making hybriodmas secreting specific antibodies. Nature (London) **276**:269–270.
11. **Sompayrac, L. M., and K. J. Danna.** 1981. Efficient infection of monkey cells with DNA of simian virus 40. Proc. Natl. Acad. Sci. U.S.A. **78**:7575–7578.
12. **Stow, N. D., and N. M. Wilke.** 1976. An improved technique for obtaining enhanced infectivity with herpes simplex virus type 1 DNA. J. Gen. Virol. **33**:447–458.
13. **Yamaizumi, M., A. L. Horwich, and F. H. Ruddle.** 1983. Expression and stabilization of microinjected plasmids containing the herpes simplex virus thymidine kinase gene and polyoma virus DNA in mouse cells. Mol. Cell. Biol. **3**:511–522.
14. **Zetterberg, A., and W. Engstrom.** 1981. Mitogenic effect of alkaline pH on quiescent, serum starved cells. Proc. Natl. Acad. Sci. U.S.A. **78**:4334–4338.