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ELECTRIC FIELD MEDIATED GENE TRANSFER

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SUMMARY: A simple physical chemical method of transferring genes into eucaryotic cells is described. Electric impulses in the intensity range of 5-10 kV/cm with a duration of 5-10 μs were found to appreciably increase the uptake of DNA into cells. After electric field treatment, stable transformants were obtained in a system containing mouse cells deficient in thymidine kinase and a plasmid DNA harbouring the Herpes simplex thymidine kinase gene. The efficiency of transformation well compares with the results of biochemical methods of gene transfer. The electric field technique appears unique in its ease and simplicity.

INTRODUCTION

A number of different biochemical methods has been developed to artificially transfer DNA into eucaryotic cells. These are: incubation of DNA-calcium phosphate precipitate with target cells; direct injection of DNA into cells, and the application of liposomes as vehicles (1-7).

This report shows that short electric field pulses are able to mediate gene transfer into eucaryotic cells which results in the expression of the transferred gene in the recipient cells. A mouse cell line deficient in thymidine kinase activity and a plasmid harbouring the Herpes simplex virus thymidine kinase gene were used for these experiments.

A mixture of mouse cells, LM(TK$^-$) (8), and plasmid DNA was subjected to electric impulses and then incubated in a selection medium. In this medium only those cells survive into which the

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DNA has penetrated. The results of this study show that the electric field technique is an easily applicable and very efficient method.

MATERIAL AND METHODS

Material. Mouse cells, LM(TK$^-$) (8), were grown in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal calf serum. The cells were trypsinized, washed, and resuspended in phosphate buffered saline (PBS; 140 mM NaCl, 2.7 mM KCl, 6.5 mM $Na_2HPO_4 \cdot 2H_2O$, 1.5 mM KH_2PO_4, pH 7.0 at 20°C) at a fincal cell density of 10^6 cells per ml. Plasmid pTK2 (9), obtained from Dr. C. Weissmann (Technical University, Zurich), is a plasmid having the Herpes simplex virus thymidine kinase gene (3.4 kb) inserted at the BamHI site of plasmid pBR322. DNA of plasmid pTK2 was prepared as described in ref. (6).

Electric field method. The electric impulse method is similar to that used for the induction of cell fusion of Dictyostelium c. (10). The apparatus is described elsewhere (11). Exponentially decaying electric impulses of an initial intensity between 5 to 10 kV/cm and a field decay time of about 3 µs were applied to a measuring cell with plane-parallel stainless steel electrodes filled with 0.5 ml of the DNA-target cells mixture. Due to the construction of the measuring cell, only 0.2 ml are exposed to the electric field. After 3 successive pulses, 0.1 ml aliquots were transferred directly to a 25 cm^2 culture flask containing 7 ml of DMEM medium. After incubating at 37°C for 24 hrs, 7 ml of 2xHAT medium (12) were added into each culture flask to select for stable transformants. Medium was changed after 3 days with 1xHAT. The number of thymidine kinase positive transformants were counted after 2 weeks.

RESULTS AND DISCUSSION

The electric field method of gene transfer is based on the observation that electric impulses above a certain threshold field strength induce transient structural changes in the membranes of vesicular and cellular systems (11,13). Material exchange is strongly enhanced through the perturbed membrane parts. For reasons of diffusion the DNA should be adsorbed on the cell surfaces before the application of electric impulses finally leads to an appreciable enhancement of DNA uptake.

In order to ensure the extensive adsorption of the plasmid DNA molecules on the mouse cell surface, the degree of binding of radioactivelly labelled DNA was measured as a function of

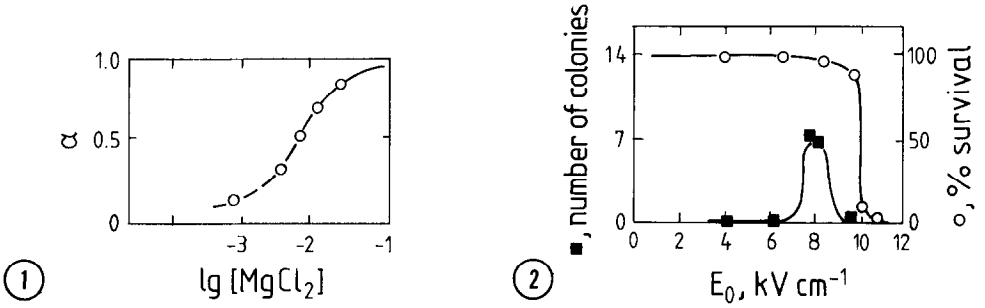

Figure 1: The degree, α, of DNA binding to mouse cells, LM(TK⁻), (≈ 10^6 cells/ml; 10 μg DNA/ml) as a function of the $MgCl_2$ concentration; PBS buffer, pH 7, 20° C.

Figure 2: The dependence of the electric field effects on the initial field intensity E_o of exponentially decaying electric fields (decay time constant of 5 μs). o, survival numbers after 3 successive electric pulses; ■, number of colonies per 1.2×10^5 pulsed cells, 10 μg DNA/ml, relative to the unpulsed control (±1 colony).

$MgCl_2$ concentration. As expected, the Mg ions facilitate the adsorption of DNA to the cell surface, the optimal concentration being close to 20 mM, as shown in Fig. 1.

It is well known that extensive application of electric impulses may finally cause lysis of protoplasts and spheroplasts (14). In order to evaluate possible destructive effects of high electric impulses on cells, the mouse cells were subjected to successive electric impulses with increasing field intensity. The field effect was determined by the amount of foams that appeared on the surface of the electrode; additionally, the cells were examined under microscope. Marked destructive effect of electric impulses on the mouse cells was observed at $\geq$ 10 kV for 3 pulses at 20° C. Since at about 20 mM $MgCl_2$ the degree of DNA adsorption onto cells is close to saturation, this Mg concentration was chosen to measure the number of stable transformants as a function of the strength of the electric field. As shown in Fig. 2, at about 8 kV/cm, there is a maximum. Compared to the unpulsed sample, the pulsed sample has 8(±1) colonies per 1.2×10^5 pulsed cells, or the yield

is 67 ($\pm$8) stable transformants per 10^6 cells per µg DNA. This value compares well with the results of biochemical methods (1-7). Although the electric field method is presently not optimized with respect to the concentrations of Mg ions, of DNA and the cell density, this physical method appears unique in its ease and simplicity.

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