Electrically induced DNA uptake by cells is a fast process involving DNA electrophoresis

V. A. Klenchin, S. I. Sukharev, S. M. Serov,* L. V. Chernomordik, and Yu. A. Chizmadzhev
Frumkin Institute of Electrochemistry, USSR Academy of Sciences, Moscow; and *Engelhardt Institute of Molecular Biology, USSR Academy of Sciences, Moscow, USSR

ABSTRACT Simian Cos-1 cells were transfected electrically with the plasmid pCH110 carrying the β-galactosidase gene. The efficiency of transfection was determined by a transient expression of this gene. When the plasmid was introduced into a cell suspension 2 s after pulse application, the transfection efficiency was shown to be $<1\%$ as compared with a prepulse addition of DNA. Addition of DNAase to suspension immediately after a pulse did not decrease transfection efficiency, thus the time of DNA translocation was estimated to be <3 s. The use of electric treatment medium, in which the postpulse colloid-osmotic cell swelling was prevented, did not affect the transfection efficiency. These results contradict both assumptions of free DNA diffusion into cell through the long-lived pores and of involvement of osmotic effects in DNA translocation. Transfection of cells in monolayer on a porous film allowed creation of the spatial asymmetry of cell-plasmid interaction along the direction of electric field applied. A pulse with a polarity inducing DNA electrophoresis toward the cells resulted in the 10-fold excess of transfection efficiency compared with a pulse with reverse polarity. Ficoll (10%) which increases medium viscosity or Mg^{2+} ions (10 mM) which decrease the effective charge of DNA, both reduced transfection efficiency 2–3-fold. These results prove a significant role of DNA electrophoresis in the phenomenon considered. The permeability of cell membranes for an indifferent dye was shown to increase noticeably if the cells were pulsed in the presence of DNA. This indicates a possible interaction of DNA translocated with the pores in an electric field, that results in pore expansion.

INTRODUCTION

Transfection of cells using treatment with pulses of high-intensity electric field is termed electrotransfection or transfection by electroporation. First demonstrated on mouse fibroblasts by Neumann et al. (1982), this phenomenon at present is widely used in molecular biology and genetics because DNA can be transfected into virtually any prokaryotic and eukaryotic cells simply, reproducibly, and at high efficiency (see the review by Potter, 1988). High efficiency for each cell type can be achieved by selecting a series of parameters the most important of which are pulse amplitude and duration (Chu et al., 1987; Dower et al., 1988).

The exact mechanism by which an electric field induces the DNA uptake by the cells is unknown, therefore electrotransfection parameters are optimized empirically only and the further increase in efficiency of the method is rather problematic. At the same time, in many works it would be highly desirable to achieve as high efficiency of transfection as possible. These are, for instance, screening of cDNA libraries in mammalian cells based on the gene product functional activity (Lee et al., 1985) and approaches to gene therapy (Titomirov et al., 1991; Felgner and Rhodes, 1991). Investigations of the mechanism of this process, i.e., understanding of the "motive forces" of electrically induced DNA translocation through the cell membrane will rationalize the choice of electrotransfection parameters and permit an increase in the efficiency of transfection.

Treatment of cells with a high-intensity electric field leads to the formation of structural defects in the cell membrane—electropores (Kinosita and Tsong, 1977; Neumann, 1989). The diameter of the electropores depends on the parameters of the pulse, which can be chosen such that the pores can reseal and, thus, not to cause cell death. Pore diameter during the pulse was determined to be up to 10 nm by the permeability for several compounds (Sowers and Lieber, 1986) and that of the long-lived pores does not exceed 1 nm (Kinosita and Tsong, 1977; Schwister and Deuticke, 1985). The extent of cell membrane poration and the transfection efficiency were found to be highly correlated as determined by entrapment of a low-molecular-weight dyes (Presse et al., 1988; Sczakiel et al., 1989). Several different mechanisms of electric field-induced translocation of DNA across the membrane were proposed: (*a*) DNA simply diffuses through the pores formed (Neumann et al., 1982; Toneguzzo and Keating, 1986) or moves along the membrane surface to the pore (Xie et

Address correspondence to Sergei I. Sukharev at University of Wisconsin, Laboratory of Molecular Biology, 1525 Linden Drive, Madison, WI 53706. Dr. Chernomordik's present address is LTPB/NICHD, National Institutes of Health, Bldg. 10, Room GC101, Bethesda, MD 20892.

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al., 1990); (*b*) transfer of DNA with the flow of water occurring due to the colloid-osmotic disbalance between the cells and the medium after transient electropermeabilization of plasma membrane (Stopper et al., 1987); (*c*) transfer of DNA as a polyanion by the action of electrophoretic force (Winterbourne et al., 1988; Taketo, 1989; Andreason and Evans, 1989; Chernomordik et al., 1990); (*d*) electrically induced trapping of DNA in the endosome-like vesicles (Chernomordik et al., 1990).

In the present paper using transient expression of β-galactosidase gene in Cos-1 cells we show that electrically induced cellular DNA uptake is a short-term process in which DNA electrophoresis plays an important role.

MATERIALS AND METHODS

Cells and plasmid

We used plasmid pCH110 (Pharmacia-LKB, Uppsala, Sweden) containing gene *lacZ* and SV40 *ori* and simian cells Cos-1 in which the amplification of SV40 *ori*-containing DNA takes place (Gluzman, 1981). The cells were grown in DMEM medium with 10% fetal calf serum (FCS; Gibco, Grand Island, NY).

Transfection in suspension

If not mentioned otherwise, the conditions were as follows. Cells (5×10^5) and plasmid (1–3 μg) in phosphate-buffered saline without calcium and magnesium (PBS) were introduced into a 60-μl cuvette, incubated 10 min at room temperature and then treated with one square pulse of field intensity 3.5 kV/cm and duration 100 μs. After the following incubation for 10 min, cells were suspended in DMEM with 10% FCS and transferred to a Petri dish (60 mm diam).

Transfection in monolayer

Cells grown on a porous film were electrically treated as described earlier (Sukharev et al., 1990; Klenchin et al., 1990) with the difference that the supporting material was wrapping cellophane. Round pieces of film (14 mm diam) were cut and washed with hot ethanol, then with water. The films were sterilized by boiling in water and used without any further treatment. The films were put into the wells of a 24-well plate and 3×10^5 cells in 1 ml medium were added. After 24 h the films with the ~75% confluent monolayer formed were rinsed with PBS and fixed in the chambers. Then 10 μg DNA in 200 μl PBS each were added and the cells were treated with a single pulse of field intensity 1.5 kV/cm and duration 100 μs. After a 10-min incubation, 1 ml of DMEM with serum was added, the cells were allowed to stay for 30 min, then removed with trypsin and seeded on Petri dishes.

Electropermeabilization assay

The dyes Lucifer Yellow (LY), fluorescein isothiocyanate-dextran (FD, 20 kDa) and propidium iodide (all from Sigman Chemical Co., St. Louis, MO) were used in different cases. To elucidate the effect of Mg^{2+} on electropermeabilization, 50–100 μl of cell suspension (10^6 cells) was supplemented with 0.2 mM FD, then pulsed and 10 min later mixed with 300 μl of growth medium. After 30 min incubation at room temperature, the cells were washed three times with DMEM and two times with PBS. Fluorescence was measured at 488 nm excitation and 520 nm emission wavelengths, respectively, with the JY-3D (Jobin-Yvon, Longjumeau, France) spectrofluorimeter. The extent of membrane poration in the presence of plasmid pCH110 (7.2 kb, 10 μg/ml in the probe) or equimolar concentration of phage λ DNA (48 kb, 67 μg/ml) was assayed with LY, which was added to a final concentration of 2 mM 5 min after cell pulsation, by the same procedure. The wavelengths of 428 and 540 nm, respectively, were set in this case. To estimate the extent of poration of cells in monolayer, 1 min after the treatment propidium iodide (150 μg/ml) was added to the chamber and incubated for 10 min. The piece of film was washed with PBS and the pictures of cells were taken using a fluorescent microscope. Then the percentage of brightly stained cells was counted.

Determination of transfection efficiency

The percentage of cells expressing *lacZ* was determined by histochemical technique according to MacGregor and Caskey (1988). Transfection efficiency (TE) was determined as a specific activity of β-galactosidase (per viable cell), which in turn was assayed by a standard colorimetric method 48 h after the experiment. TE was expressed by formula $TE = 10^6 \times (OD_{420} - OD_{550})/(N \times T)$, where OD_{420}, OD_{550} are optical densities at wavelengths of 420 and 550 nm; N is the number of cells; T is the time of incubation in hours (An et al., 1982). The values presented are the means of two experiments with at least two parallel assays, the variance being no more than 25%. Cell viability was expressed as percentage of the number of cells, attached to the plastic 48 h after the experiment, from the number of untreated cells in parallel control. Usually, the cell viability after pulse treatment was ~30–50%.

RESULTS

Expression of β-galactosidase gene inserted into the plasmid pCH110 in Cos-1 cells is preceded by the stages of DNA translocation through the plasma membrane, its migration to the nuclei and amplification. We focused on the first stage, thus we had to prove that in a given range of plasmid concentration the amount of plasmid penetrated is reflected correctly by the activity of expressed enzyme. Linearity of TE dependence within the range of DNA concentrations up to 50 μg/ml (Fig. 1) supports the assumption that if the amount of penetrating DNA is proportional to its external concentration, then with constant conditions an equal portion of penetrating molecules is expressed. The number of transfected cells counted after histochemical staining in this experiment ranged from 0 to 22%. The distinct difference in staining deepness of different cells in one preparation was revealed. In all the following experiments only the bulk colorimetric method of TE assaying was imployed.

If DNA penetrates into the cell through long-lived pores, then its addition to the cells after a pore-forming pulse should also lead to transfection. The results of the runs where DNA was introduced into suspension at

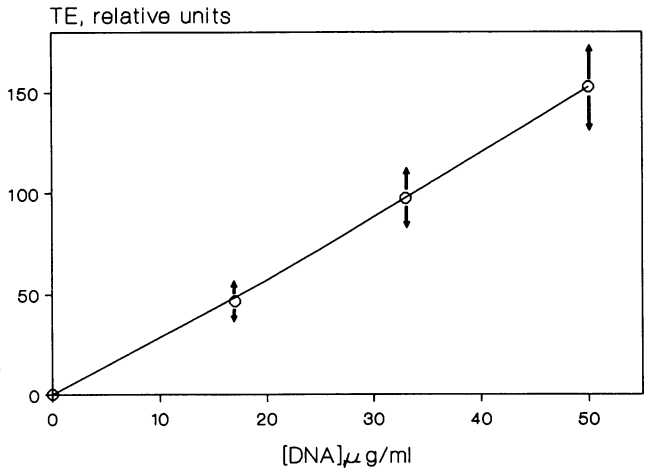

FIGURE 1 Transfection efficiency of Cos-1 cells as a function of plasmid concentration in cell suspension. Plasmid pCH110 containing β-galactosidase (*lacZ*) gene was introduced to cell suspension 10 min prior to pulse delivery. Pulsation medium consisted in PBS without calcium and magnesium. Square pulse parameters: $E = 3.5$ kV/cm, $t =$ 100 μs. 10 min after pulse application cells were transferred to growth medium and plated on Petri dishes. All the manipulations carried out at room temperature. Transfection efficiency (TE) was determined 48 h after, as described in Materials and Methods.

various moments both before and after the pulse are shown in Fig. 2. The efficiency of penetration of DNA added after the pulse is two orders of magnitude lower than in the case of the DNA added before the pulse.

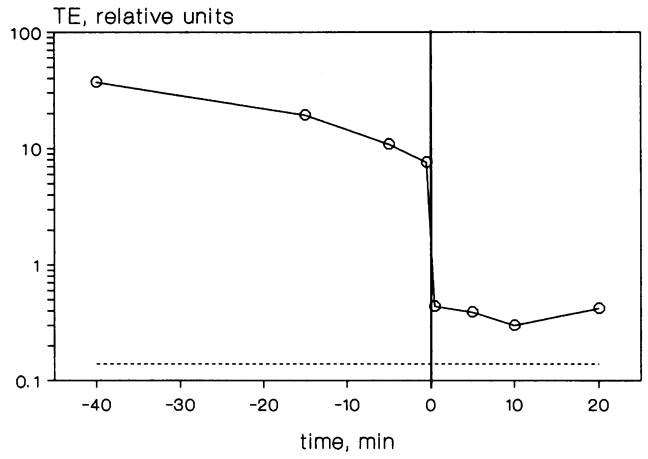

FIGURE 2 Dependence of transfection efficiency of Cos-1 cells on the time of DNA addition. The plasmid was introduced into cell suspension at different moments before (*left part of the plot*) and after (*right part*) pulse application. The moment of pulse delivery is denoted as the time zero. The background level of β-galactosidase activity in cells not subjected to the pulse is shown by dashed line. Other experimental conditions as described in the legend to Fig. 1.

Under the same conditions, the permeability of cells for LY decreased twofold within 20 min (data not shown). At the same time, the transfection efficiency of the post-pulse addition of plasmid exceeded by 2–3-fold the value without electrical treatment (which was indistinguishable from background) and within 30 min did not depend noticeably on the time of addition.

We estimated the characteristic time of plasmid penetration into the cells using plasmid accessibility for DNAase. Cell suspension with DNA was supplemented with DNAase at different times after the pulse application. As seen from the data of Fig. 3, when DNAase was introduced 2 s before the pulse, the transfection efficiency decreased to the background level, whereas addition of DNAase 2 s after the pulse did not decrease transfection efficiency as compared with the control. This indicates that all transfecting DNA becomes inaccessible for DNAase, i.e., translocated into the cells in 3 s, or less after the electric treatment.

To investigate the influence of colloid-osmotic process after pulsation on electrotransfection we performed an experiment in conditions that prevented cell swelling (Zimmermann et al., 1976). Inulin was added in the pulsation medium to a concentration of 20 mM. There was no difference observed in TE compared with the control (data not shown).

To elucidate the role of electric field direction we used a monolayer of cells on a porous film for transfection (Fig. 4), as briefly described earlier (Klenchin et al., 1990). In this system mainly the upper surface of the cells growing in a monolayer is accessible to DNA. The electric field normal to the monolayer plane induced DNA electrophoresis either toward or away from the

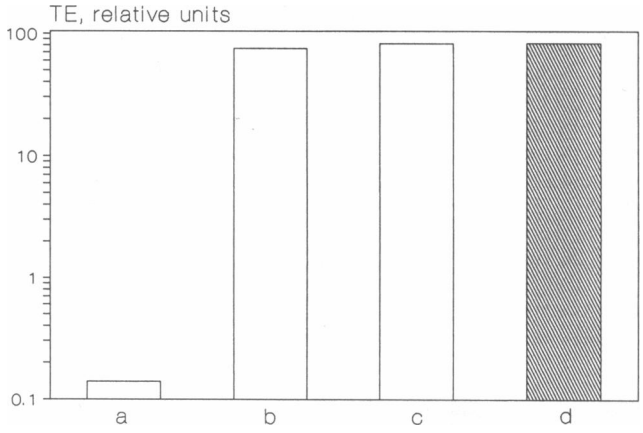

FIGURE 3 Effect of DNAase on transfection efficiency of Cos-1 cells. DNAase (to 5 mg/ml) and $MgCl_2$ (to 5 mM) were added to cell suspension 3 s before (*a*), within 3 s (*b*), and 20 s after (*c*) the pulse delivery; (*d*) only $MgCl_2$ added. Other experimental conditions as described in the legend to Fig. 1.

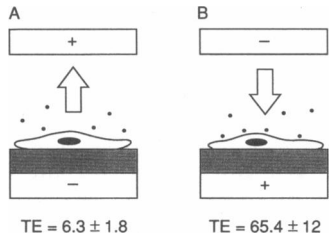

FIGURE 4 (*Top*) Schematic presentation of an experimental system for studying the role of electric field direction on electrotransfection. The spreading cells are grown on porous film impermeable for DNA. When the DNA is added to cell monolayer, the upper cell surface only is accessible for it. Electrotransfection is induced by pulsed electric field directed normally to the cell monolayer. Depending on polarity, the electric pulse will cause DNA electrophoresis away from (*A*) or toward the cells (*B*). (*Bottom*) The results of TE assay after transfection with single pulses of each polarity. Cells were seeded on the cellophane films 24 h before experiment and formed 75% confluent monolayer. The films with cells were rinsed in PBS and fixed in a special chamber (see Sukharev et al., 1990), then the plasmid (6 μg in 200 μl PBS) was added. 10 min after the pulse (2.5 kV/cm; 100 μs) of appropriate polarity was applied. TE was determined as described in Materials and Methods.

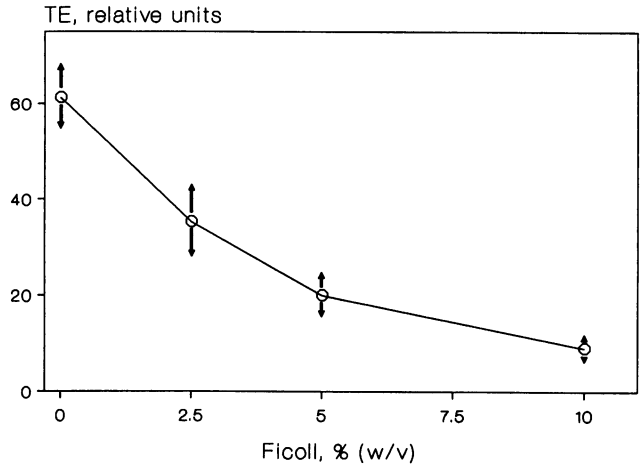

FIGURE 5 Transfection efficiency as a function of Ficoll concentration in the medium. DNA and Ficoll were introduced into cell suspension 10 min and 2 min before pulse application, respectively. Other experimental conditions as described in the legend to Fig. 1.

cells, depending on the polarity of the pulse. The data of Fig. 4 (*bottom*) indicate that when DNA moves towards the cells (lower electrode is positive), transfection efficiency is one order of magnitude higher than with a reverse polarity. Permeability of the cells for propidium iodide, added 1 min after the pulse, was the same at both pulse polarities. This indicates that the degree of membrane electroporation does not depend on the direction of electric field. We failed to detect any significant difference in cell viability between both polarities. In either case cell survival was ~40%.

In two further experiments we investigated the effects of factors that decrease the electrophoretic mobility of DNA in the pulsation medium. Fig. 5 plots transfection efficiency versus Ficoll-400 concentration in the medium, which noticeably increases the viscosity of the medium (Osterman, 1984) and, thus, decreases the electrophoretic mobility of DNA. Other factors that determine the electrophoretic mobility of DNA in solution are: the effective charge of the molecule and the ionic environment. Effect of Mg^{2+} on transfection efficiency is shown in Fig. 6. As it could be expected, both factors, such as Mg^{2+} binding to DNA (reduces charge) and rise of ionic strength (increases shielding), contribute to the decrease of TE. In this experiment DNA was added 10 min before the pulse. The same relative decrease was also observed when the time of prepulse incubation of cells with DNA and Mg^{2+} was reduced to several seconds (data not shown). Interestingly, the presence of Mg^{2+} ions had no effect on electrically induced permeability of cells for FD, while the cell viability rose 1.5–2-fold.

The special experiment has shown that in a high electric field the DNA may interact with the membrane. As a criterion we have chosen cell membrane permeability for indifferent dye after pulse delivery. We measured the amount of Lucifer Yellow (LY) which entered the cells. The dye was added 5 min after the electric treatment of cells in the presence or absence of DNA. The data of Fig. 7 indicate that in the presence of

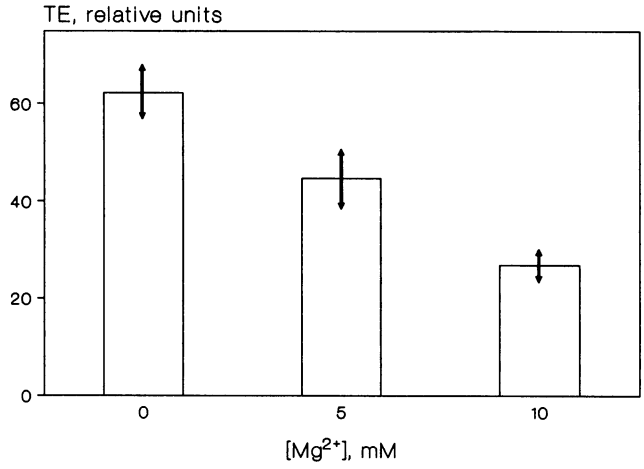

FIGURE 6 Effect of $MgCl_2$ on the efficiency of transfection. Suspension of Cos-1 cells was supplemented with $MgCl_2$ to appropriate concentration, then DNA was added and 10 min after the electric pulse was applied. (*a*) No $MgCl_2$; (*b*) 5 mM $MgCl_2$; (*c*) 10 mM $MgCl_2$. Other experimental conditions as described in the legend to Fig. 1.

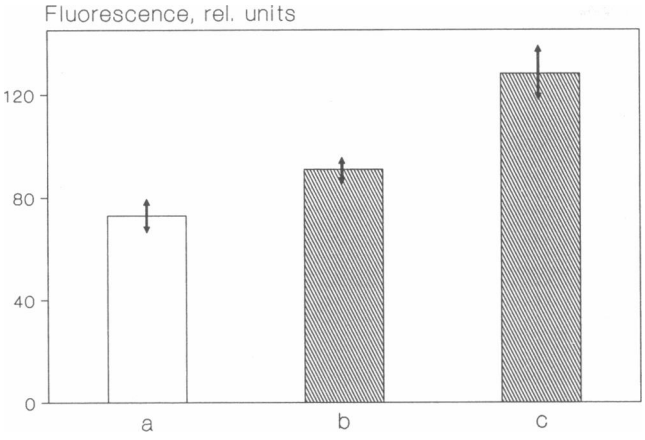

FIGURE 7 Increase in cell membrane permeability for Lucifer Yellow (LY) after electric field treatment in the presence of DNA of different size. 1.5 × 10^6 Cos-1 cells in 100 μl PBS were subjected to 3.5 kV/cm, 100 μs pulse in the presence of equimolar concentration of plasmid pCH110 (7.2 kb) or phage λ DNA (48 kb) and 5 min after LY was added to 3 mM concentration. After subsequent incubation for 10 min, 300 μl of growth medium with serum was added and the cells were allowed to stay for 30 min at 25°C (resealing time). Then the cells were washed carefully and the amount of LY entrapped was measured. See Materials and Methods for details. (*a*) No DNA in the sample; (*b*) pCH110, 10 μg/ml; (*c*) phage λ DNA, 67 μg/ml. Fluorescence intensity of control cells not subjected to electric field is taken as zero.

plasmid pCH110 (7.2 kb) the entry of LY increased. The effect on LY uptake was larger in the presence of an equimolar amount of phage λ DNA (48 kb). Hence, the larger the size of DNA is, the stronger the effect should be on the postpulse permeability of the membrane.

DISCUSSION

The level of transient expression (TE) of the β-galactozidase in our system is equal to the product of the number of cells expressing the gene and the mean level of expression in each such cell. Because the level of expression in one cell may vary substantially, the TE value determined by bulk colorimetric technique, which accounts for both factors, reflects the rate of DNA uptake more adequately than the percentage of stained cells. TE represents *lacZ* expression normalized to cell survival, thus the effect of cell killing by a high electric field (Chu et al., 1987; Sczakiel et al., 1989) can be neglected.

The peculiar properties of Cos-1 cells provided some advantages to the experimental system: high sensitivity due to plasmid amplification and an easy way of assaying cell survival. Because 24 or 48 h after electric treatment the dead cells detach, they can be simply washed away from the living cells, which stick to the bottom of the dish. The linear dependence of transfection efficiency on DNA concentration (which is inherent to electrotransfection: Toneguzzo and Keating, 1986; Chu et al., 1987), is not distorted by plasmid amplification in these cells (Fig. 1), hence, one can be sure that the specific activity of the enzyme linearly reflects the amount of DNA entering the cells.

The data shown in Figs. 2 and 3 prove that the process of plasmid penetration is completed within 3 s after the pulse delivery. Indeed, when the DNA is introduced to the cell suspension after pulse application, an insignificant fraction (<1%) is able to enter the cells. At the same time, in the case of prepulse DNA introduction practically all the DNA, which undergoes expression, in a couple of seconds after the pulse becomes inaccessible for DNAase. These data are consistent with results obtained by Xie et al. (1990) and Taketo (1988) on *Escherichia coli* transformation, who reported drastic decrease in efficiency when DNA is added following pulse delivery. All the results described above suggest that the DNA may be transferred into cells within very short period of time, directly during the pulse applied. This assumption is reasonable just because there are significant forces acting during the electric pulse, which may drive DNA molecules across the membrane. These are electrophoretic force and electroosmotic water flux (Sowers, 1988; Dimitrov and Sowers, 1990) occurring near charged edges of the membrane pore (see Fig. 8).

In the present work we have proposed a simple system that allowed us to create a spatial asymmetry of DNA around each cell (Fig. 4) and reveal the essential role of

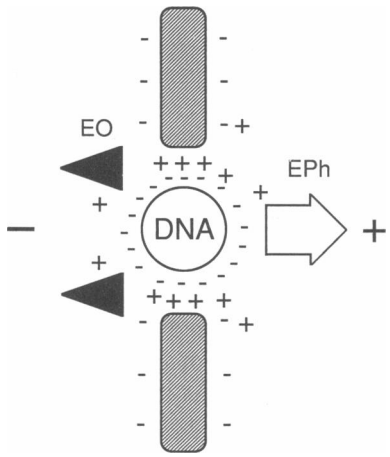

FIGURE 8 The scheme illustrating DNA movement through the hydrophilic pore in the membrane. Electrophoretic force (EPh) moves DNA toward the positive electrode, while the electroosmotic water flux (EO) resulted from the movement of counterions along the negatively charged membrane surface is directed oppositely.

electric field direction in cell transfection. We have found a 10-fold increase in transfection efficiency with a polarity causing DNA electrophoresis toward the cells, as than with an inverse polarity. The hypothesis of DNA movement with the electroosmotic water flux cannot explain the fact we observed, since water fluxes in opposite direction, i.e., toward the negative electrode (see Fig. 8). The noticeable level of transfection at the reverse polarity can be explained by the nonideal asymmetry of DNA in a real system. Preliminary experiments showed that the degree of real asymmetry, i.e., the accessibility of the bottom side of the cells for DNA depends on the adhesive properties of the film and on the monolayer density. In similar sets of experiments with less adhesive films, we obtained only threefold difference in TE with the two opposite polarities (Klenchin et al., 1990).

When the electrophoretic mobility of DNA is diminished by an increase in medium viscosity, as well as by a decrease of the polyanion's effective charge (and/or by more effective ionic shielding), the transfection efficiency is also reduced (Figs. 5 and 6). Note, in the presence of Ficoll TE decreases by about the same factor as respective concentrations of Ficoll increase the viscosity of aqueous solutions (Osterman, 1984). Because the electrophoretic mobility inversely depends on the viscosity of the medium, this result allows us to assume that a significant part of the plasmid penetrates to the cell directly from the bulk, because the motion of molecule bound to the cell surface should be affected less by viscosity of the bulk solution.

The effect induced by adding Mg^{2+} could, in principle, be due to a decrease of electroporation in the presence of Mg^{2+}. This is indicated by a sharp increase in the cell viability in the presence of Mg^{2+}. However, as mentioned in Results, permeability of electric field-treated cells for FD did not change in a given range of Mg^{2+} concentrations. The decrease of TE can also be explained by the enhanced DNA adsorption in the presence of Mg^{2+} ions. The adsorbed DNA is retained on the cell surface and does not penetrate into the cell (Wong and Neumann, 1982). However, the efficiency of transfection in the presence of magnesium ions drops to the same extent when the time of prepulse incubation of DNA with cells is 3–5 s. On the other hand, the Mg^{2+}-dependent binding of DNA on mammalian cells takes several minutes (Belyaev et al., 1988). Therefore, we believe that magnesium decreases transfection efficiency by decreasing the DNA electrophoretic mobility.

It has been shown that at the optimal electric field intensity corresponding to a given pulse duration the yield of stable transformants is always higher when using longer pulses (Kubiniec et al., 1990). The electrophoretic transfer is the same at equal values of $E \times \tau$ product (E, electric field intensity; τ, pulse duration), whereas the cell survival after the electrical treatment depends on E stronger than on τ (Montane, 1989). Andreason and Evans (1989) showed that using a pulse of high amplitude and small duration (8 kV/cm, 15 μs) transfection was inefficient and cell viability dropped to 50%. However, the treatment by the same pulse followed by 12 pulses of very low amplitude gave a more than 20-fold rise of transfection efficiency. Electrophoretic introduction of DNA is just the assumption that Andreason and Evans made.

The electrophoretic movement of DNA molecules toward the cells has to cause a certain increase of the DNA concentration near the membrane surface. Then the DNA has to be translocated through the membrane. Because the characteristic time of this process was found to be not more than 3 s after pulse delivery, the probability of DNA diffusion through long-lived pores is very low. Jastreboff et al. (1987) and Knutson and Yee (1987) have shown that even huge DNA molecules can be readily introduced into cells by electrotransfection (more than 65 kb and 150 kb, respectively). The diameter of the statistical coil of such DNA is estimated being more than 1 μm. Comparing this value with the relatively small sizes of pores ($\sim$10 nm; Sowers and Lieber, 1986) we may exclude the significant role of the DNA free diffusion through the short-lived electropores.

In principle, DNA can be drawn into cell with the water flux during colloid-osmotic cell swelling (Stopper et al., 1987). In addition, the cell swelling could create the membrane tension leading to pore widening and to enhancement of the DNA entrapment. The formation of huge pores after electrical treatment of erythrocytes in slightly hypotonic solution has been observed recently by Chang and Reese (1990). To check the possible role of the osmotic effects we used slightly hyperosmotic pulsation medium containing 20 mM inulin. In this case the addition of relatively large osmotically active molecules, which do not penetrate through porated membrane, prevents colloid-osmotic cell swelling upon electropermeabilization (Zimmermann et al., 1976). The absence of any changes of the transfection efficiency shows that osmotic effects do not participate directly in the phenomenon. More likely DNA crosses the plasma membrane by the action of electrophoretic force.

Concerning possible structural changes of the membrane, which may mediate DNA transfer, there are at least two possibilities. (*a*) Electropores. When pores in the membrane are formed, electric current begins to flow through the cells and electric field lines concentrate in the pores. In other words, electric field strength near and inside the pore is higher than in the bulk, by this reason at the appropriate field polarity, DNA is to be drawn into the pore. It should be stressed, this peculiar-

ity is a significant advantage for an electrophoretic mechanism in contrast to diffusional one with no preferential direction of DNA movement. Due to very high field intensity in the pore one can expect the effect of plasmid orientation (Diekmann et al., 1982) and a subsequent fast passing of DNA through the pore. Because neither DNA molecule nor pore in the membrane have a rigid structure, we suggest that even if the size of DNA molecule is larger than that of pore, then the pore may widen as a result of their interaction and pass the DNA through (see Fig. 9 *A*). As seen from Fig. 7, membrane permeability for indifferent dye (LY) after electric treatment in the presence of DNA is markedly higher than without it. We can exclude penetration of this dye in complex with DNA because in this set LY was added 5 min after pulse application, when DNA entrapment is completed. The larger the size of DNA molecule, the higher the membrane permeability, i.e., extent of poration. This observation confirms the assumption that DNA may interact with the pore by the action of high electric field, that results in additional pore expansion. However, one can expect formation of some specific DNA-permeant structures induced by DNA itself, the traces of which we have detected.

(*b*) Endocytosis-like entrapment. A work on electric field-stimulated trapping of DNA by liposomes (Chernomordik et al., 1990) showed the penetration of DNA into the inner liposome volume not through the pores but as a component of a DNA-containing membrane vesicle. It is assumed that the electrophoretic force facilitates the formation of an invagination which may bud off inside the liposome (see Fig. 9 *B*). Whether this variant takes place in the case of cell electrotransfection is not clear. A certain role in electrotransfection played by macroscopic changes in the membrane structure evoked by the electric field is not to be ruled out (Escande-Geraud et al., 1988; Gass and Chernomordik, 1990).

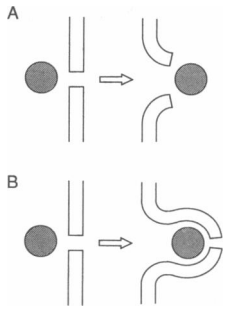

FIGURE 9 Two possible ways of DNA-membrane interaction induced by high electric field. First DNA molecule moves along the electric field lines towards the primary pore. Then DNA may widen the pore and pass through directly to the cell interior (*A*). Otherwise, DNA and membrane form endosome-like structure (*B*), which may bud off inside the cell.

To sum up the consideration of the data presented above we can say that the high electric field appears to be a unique factor providing fast introduction of foreign DNA into cells. Electric field causes not only reversible membrane poration, but also concentrates DNA near the membrane and directs DNA toward the primary pores by electrophoresis. Interaction of DNA with the membrane in a high electric field may also induce specific permeant structures to develop. The nature of these structures needs to be clarified.

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