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Study of Mechanisms of Electric Field-Induced DNA Transfection. V. Effects of DNA Topology on Surface Binding, Cell Uptake, Expression, and Integration into Host Chromosomes of DNA in the Mammalian Cell

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ABSTRACT Neumann and coworkers (Neumann, E., M. Schaefer-Ridder, Y. Wang, and P. H. Hofschneider. 1982. *EMBO J.* 1:841–845) have shown that the efficiency of pulsed electric field (PEF)-induced DNA transfection of mouse L-cells by the thymidine kinase gene is several times higher for the linear DNA than for the closed circular DNA. Transfection of *Escherichia coli* bacteria by several plasmids indicates that the transfection efficiency was much higher for the closed circular/supercoiled (sc-) and circular/relaxed (cr-) DNA than for the linearized (ln-) DNA (Xie, T. D., L. Sun, H. G. Zhao, J. A. Fuchs, and T. Y. Tsong. 1992. *Biophys. J.* 63:1026–1031). To resolve these conflicting observations, we have systematically examined electrotransfection of NIH3T3 mouse fibroblast by the plasmids, $pRSV_{cat}$, $pRSV_{neo}$, and $pRSV_{gpt}$. Mg^{2+}-facilitated surface binding of DNA before, and DNA uptake by 3T3 cells after treatment with PEF, were monitored by ^3H-labeled plasmids. Transfection efficiency was evaluated by both the transient expression of chloramphenicol acetyltransferase (cat) activity 2–3 days after, and the permanent expression of neomycin phosphotransferase (neo) and xanthine-guanine phosphoribosyltransferase (gpt) genes in the transformants 2 weeks after the PEF treatment. Our results indicate that cell surface binding and PEF-induced cell uptake of DNA did not depend on the topology of DNA. However, both the transient and the permanent expression of the plasmids were three to five times more efficient for the cr-DNA and the sc-DNA than for the ln-DNA. These results indicate that electrotransfection of cells involves several steps: the cation-dependent binding of DNA to the cell surface, the electric field-driven DNA entry into the cells, the transient expression of DNA, and the integration of DNA into the host chromosomes. For understanding mechanisms of electrotransfection, only the DNA binding to the cell surface and the electric field assisted membrane-crossing of DNA are relevant. Both the expression of the loaded DNA and the DNA integration into the host chromosomes depend more on the properties of the cell and its interactions with a foreign gene. Since these properties and interactions will be similar irrespective of the method chosen to facilitate DNA transfer, they are not relevant for the study of mechanisms of electrotransfection. Our results also support the idea that the PEF-induced cellular uptake of DNA is mainly by the electrophoresis of the surface bound DNA across the plasma membrane.

INTRODUCTION

There has been a considerable interest in the mechanism of electric field-induced DNA transfection of cells for the past several years (Chang et al., 1992; Foster and Neumann, 1989; Tsong, 1991). This interest among cell biologists is largely attributed to several recent developments. First, from the mechanistic point of view, it remains a mystery how molecules as large as DNA can enter a cell by a pulsed electric field (PEF) without a simultaneous loss of the cytoplasmic macromolecular content, or severely injuring the cells as to lead to cell death (Kinosita and Tsong, 1977b; Sowers and Lieber, 1986; Tsong, 1991). Furthermore, it has been reported that electric field too weak to instigate electroporation of cell membranes, can also facilitate DNA transfection (Tsong, 1991; Xie and Tsong, 1990). Second, from the practical point of view, electric field-induced gene transfer continues to be a method preferable over other methods for application in cellular and molecular biology, because it is relatively simple to perform and it does not require expensive equipment (Chang et al., 1992; Foster and Neumann, 1989; Tsong, 1991). Third, transfection efficiency by the PEF method has been improving steadily, and there is a likelihood that when augmented with other procedures, electrotransfection may reach the level of effectiveness practical for gene therapy (Chang et al., 1992; Foster and Neumann, 1989).

Since the successful application of the PEF method for introducing DNA into mouse L-cells by Neumann and coworkers (1982), several mechanisms have been proposed to explain the PEF-assisted DNA transfection of cells. These authors have suggested that DNA can diffuse through membrane pores created by the PEF into the cytoplasm (Newmann et al., 1982). Xie et al. (1990) then showed that the efficiency of the electrotransfection directly correlated with the amount of DNA bound to the cell surface under a given set of electric parameters, suggesting that surface diffusion of DNA through membrane pores might be a predominant pathway for the cellular uptake of DNA. Electro-osmotic flux induced by a PEF (Dimitrov and Sowers, 1990) may also carry DNA into a cell if the duration of the PEF is sufficiently long, e.g., several milliseconds or longer. Electrophoresis of DNA across cell membranes is another plausible mechanism. Klenchin et al. (1991) and Sukharev et al. (1992) have shown convincingly that electrophoresis of DNA by the applied field is the dominant pathway for the PEF-induced DNA transfection of cells. The efficiency of transfection was several fold higher if the polarity of a PEF was to drive the DNA

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toward the cells, layered on a thin film which conducts electricity, than if the polarity was to drive DNA in the opposite direction (Klenchin et al., 1991; Sukharev et al., 1992). Endocytotic transfer of DNA across the lipid bilayer has also been suggested (Chernomordik et al., 1990). DNA loaded into liposomes by PEF was inaccessible for binding by ethidium bromide, an indication that the loaded DNA was enclosed in lipid vesicles which formed by endocytosis. Other studies have shown that alternating electric fields (AC) are more efficient for electrotransfection than the direct current electric fields (DC) (Tekle et al., 1991). Low intensity AC fields too weak to cause electroporation of cell membranes can also elevate DNA transfection by as much as 10,000-fold (Xie and Tsong, 1990).

Another interesting observation of Neumann and coworkers (Neumann et al., 1982) is that the efficiency of electrotransfection of mouse L-cells by the thymidine kinase gene was much higher for the linearized (ln-) DNA than for the closed-circular/relaxed (cr-) DNA. Because the dependence of transfection efficiency on the topology of DNA might shed light on mechanisms of electrotransfection, we have undertaken a systematic investigation of effects of DNA topology on the transfection efficiency. For the transfection of *Escherichia coli* by several plasmids carrying the ampicillin-resistant gene, the PEF-induced cell uptake of plasmids was independent of DNA topology. However, the transfection efficiency was a few orders of magnitude higher for the cr-DNA and the closed-circular/super coiled (sc-) DNA than for the ln-DNA (Xie et al., 1992). In this case, the transfection efficiency was similar for cr-DNA and sc-DNA. It was subsequently shown that, lowered efficiency of the ln-DNA was due to its instability in the host cells: most of the loaded ln-DNA was degraded by the host enzymes within 3 h. Both cr-DNA and sc-DNA had much higher stability and were expressed in the host cells. These results contrasted the observation of Neumann and coworkers (1982) who have found that the transfection of mouse L-cells by thymidine kinase gene was more efficient with ln-DNA than circular DNA. It was unclear whether or not the disagreement in the two studies was due to the different mechanisms of DNA transfection in the bacterial and the mammalian cells. We report here a systematic study of the electrotransfection of a mammalian cell, NIH 3T3 fibroblast, by three topoisomers (ln-, cr-, and sc-) of the plasmids, $pRSV_{cat}$, $pRSV_{neo}$, and $pRSV_{gpt}$. Experiments with HeLa cells were consistent with these results, although only transfection with the NIH 3T3 is reported in this communication.

MATERIAL AND METHODS

Amplification, isotope-labeling, and purification of plasmid DNA

E. coli JM 105 was used to prepare the three topoisomers of DNA for this study. The plasmids, $pRSV_{cat}$ (5.1 kilobases (kb)), $pRSV_{neo}$ (5.74 kb), and $pRSV_{gpt}$ (about 5.5 kb), which carry the mammalian expression vectors for chloramphenicol acetyltransferase, neomycin phosphotransferase, and the bacterial genes xanthine-guanine phosphoribosyltransferase, respectively, were obtained from the American Type Culture (ATCC) Inc. (Data Sheet Nos. 37152, 37198, and 39199, April 19, 1989; see also Gorman et al. (1983)). These plasmids were loaded separately into *E. coli* JM105 by the electroporation method (Xie and Tsong, 1992). The cell suspensions were then plated on LB-agar solid selective culture medium (LBASSCM) which contained 10 g of Bacto-tryptone, 5 g of Bacto-yeast extract, 5 g of NaCl, 15 g of agar, 25 mg of streptomycin, and 30 mg of ampicillin per liter. After incubation overnight at 37°C, single colonies were chosen to inoculate 25 ml of LB-selective liquid culture medium (LBSLCM) in which the agar was omitted from LBASSCM. These cultures were kept in a shaker overnight at 37°C. 1 ml from each culture was then used to inoculate 500 ml of a culture medium which contained 7 g of K_2HPO_4, 3 g of KH_2PO_4, 0.05 g of sodium citrate, 0.1 g of $MgSO_4$, and 1 g of $(NH_4)_2SO_4$, per liter. To each culture was added 12.5 mg of streptomycin, 15 mg of ampicillin, 5 ml of 20% glucose, and 0.5 ml of a 4-mg/ml vitamin B1 solution, then incubated at 37°C in a shaker. At the mid-log phase (specific optical density $OD_{600\ nm}$ 0.8 – 1.0), a 2.5 ml of solution containing 34 mg/ml chloramphenicol, with or without 1 ml of [^{3}H]thymidine (1 mCi), was added. The culture was further incubated, and the plasmid was amplified in the cultured cells, at 37°C for 12–16 h. Cells were harvested and lysed with NaOH and sodium dodecyl sulfate. DNA of these plasmids (both radioactively labeled and unlabeled) were precipitated with isopropanol and ethanol and purified by ultracentrifugation in a cesium chloride/ethidium bromide density gradient. Monomeric sc-DNA (radioactively labeled and unlabeled) of these plasmids was isolated by electroelution, then purified by phenol, chloroform, and ethanol extraction. Purified DNA was dissolved to appropriate concentrations in a TE buffer (10 mM Tris buffer containing 1 mM EDTA, at pH 7.4). ^{3}H-labeled and unlabeled ln-DNA and cr-DNA were prepared from the purified sc-DNA as described previously (Xie and Tsong, 1990, 1992; Xie et al., 1990, 1992). Agarose gel electrophoresis was performed to ensure the purity and the correct topology of the DNA (Xie et al., 1992). Different topoisomeric forms of DNA banded at different positions. Monomeric forms of these isomers were identified and isolated by electroelution as described elsewhere (Sowers and Lieber, 1986; Xie et al., 1992).

NIH 3T3 cell cultures

NIH3T3 (mouse embryo fibroblasts) cell strain was obtained from the ATCC. Cells were grown as monolayer culture in the Complete Medium which contained Dulbecco's modified Eagle's medium, penicillin, and streptomycin, supplemented with 10% calf serum. Cells were maintained at 37°C in a water-jacketed CO_2 incubator (95% air and 5% CO_2). At the log phase of growth, cells were treated with trypsin/EDTA, harvested by centrifugation at 1000 *g* for 5 min at 4°C, and then washed twice with the phosphate-buffered saline which contained 137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4, pH 7.3. Pelleted cells were then resuspended in a Binding and Electroporation Medium (BEM) at a desired concentration (usually between 0.5 and 1.0×10^7 cells/ml) and maintained on ice until the experiment within 3 h. The composition of BEM is 1 mM Tris buffer, at pH 7.4, a desired concentration of $MgCl_2$ (0.25–12.5 mM), and a suitable amount of sucrose to make the solution isotonic (300 mOsm).

Binding of DNA to cell surface

0.1 μg of ^{3}H-labeled DNA (one of the three topoisomeric forms of the plasmids, $pRSV_{cat}$, $pRSV_{neo}$, or $pRSV_{gpt}$) was added to 500 μl of the NIH 3T3 cell suspension in the BEM. After mixing thoroughly, the sample was incubated on ice for 5 min and then centrifuged at 5000 *g* for 2 min. The radioactivity in the supernatant and the pellet were counted with a Packard Model 1600CA liquid scintillation analyzer (Packard Instrument Co., Inc., Downers Grove, IL). The fraction of DNA bound to the cell surface, or the DNA binding ratio (BR) was defined as the ratio of CPM of the pellet and the total CPM of the sample.

Pulsed electric field treatment

The apparatus for the PEF experiment has been described (Kinosita and Tsong, 1977a). It consists of a Cober 605 high voltage generator, a cylindrical plexiglas sample chamber with two platinized platinum electrodes, at

the two ends, in contact with the cell suspension. The two electrodes sit on two hollow stainless steel blocks, through which cooling water circulates to control the sample's temperature. The distance between the two electrodes are adjustable between 0.1 and 0.5 cm, and the volume of the sample is 30–500 μl. In most experiments, a distance of 0.25 cm and a sample volume of 150 μl was used. The square waveform was used in all experiments. The waveform and the amplitude of an electric pulse were directly monitored with a Model 5103, Tektronix storage oscilloscope (Tektronix, Beaverton, OR). The initial temperature of the sample was about 2°C. After treatment with a PEF, the temperature of a sample increased, but never exceeded 5°C, as was measured by a microthermistor probe with a time constant of 0.1 s. The temperature of the sample returned to the initial temperature in <10 s.

Assays for uptake and transient and permanent expression of DNA

An appropriate amount of plasmid DNA was added to 500 μl of cell suspension (in the BEM) and vortexed for 15 s. The mixture was then incubated on ice for 5 min. An aliquot (150 μl) was transferred into the electroporation chamber, which was maintained at 2°C. A single electric pulse of strength up to 4 kV/cm, and a width of 5 ms was applied. The sample was then transferred to a 1.5-ml test tube for DNA uptake and expression experiments. For the assay of ^{3}H-labeled DNA uptake, PEF-treated cells were first incubated at room temperature for 10 min then washed twice with phosphate-buffered saline and one time with isotonic sucrose, followed by treatment with DNase I to remove surface bound DNA (Xie et al., 1992). Cells were then spun down by centrifugation at 5000 *g* for 2 min. Radioactivity in the supernatant and the pellet was counted. Transfer ratio (TR) is defined as the ratio of CPM in the pellet divided by the total CPM of the sample.

Transient expression of the loaded plasmid, $pRSV_{cat}$, was assayed by the appearance of chloramphenicol acetyltransferase (cat) activity following the standard procedure (Kingston and Sheen, 1990). The PEF-treated cell sample was transferred into a 1.5-ml test tube which contained 1 ml of Complete Medium. After a 30-min incubation at room temperature, the sample was mixed with 10 ml of the Complete Medium in a petri dish and kept at 37°C in the water-jacketed CO_2 incubator. After 48–72 h, cells were harvested by centrifugation. After cell disruption by a freeze/thaw procedure, cat activity in the cell extract was assayed by monitoring the acetylation of [^{14}C]chloramphenicol. Chloramphenicol and acetylated chloramphenicol were separated by the thin layer chromatography on silica gel. After autoradiography, regions of the gel corresponding to chloramphenicol and acetylated chloramphenicol were scraped and counted with a scintillation counter. In our experiments diacetylated species was not detected. For our analysis, we define the Acetylation Ratio (AR) as the CPM of the two monoacetylated chloramphenicol divided by the total CPM count.

To assay for the permanent expression of a plasmid, the PEF-treated cell/DNA mixture was transferred into a 1.5-ml test tube, diluted 10 times with the Complete Medium. After incubation at room temperature for 30 min, the sample was mixed with 10 ml of the Complete Medium and kept in a petri dish for 2 days at 37°C in the water-jacketed CO_2 incubator. Cells were then transferred into a Selection Medium (liquid) specific for the plasmid and continued incubation in the water-jacketed CO_2 incubator. For the selection of neo transformants, the Selection Medium contained 400 μg/ml G418 (Geneticin). For the selection of gpt transformants, the Selection Medium contained 10 μg of mycophenolic acid, 15 μg/ml hypoxanthine, 2 μg/ml aminopterin, 10 μg/ml thymidine, 100 μg/ml xanthine, 5 μg/ml glycine, and 150 μg/ml glutamine. After 12 days of growth in the Selection Medium, the cells were washed with the Complete Medium, fixed with methanol, and stained with methylene blue to count the colonies of the transformant.

RESULTS

Mg^{2+}-dependent DNA binding to cell surface

Similar to the data obtained with *E. coli* (Xie et al., 1992), binding of [^{3}H]DNA to the 3T3 cell surface was not dependent on the topology of the DNA. Fig. 1 gives the dependence of the binding of [^{3}H]DNA to 3T3 cell surface on the concentration of $MgCl_2$. In these experiments, the cell concentration was 5×10^6/ml and the DNA concentration was 0.2 μg/ml. No difference in binding was detected for the three topological forms of DNA selectively derived from the three plasmids.

PEF-induced cellular uptake of DNA

Likewise, no differences were found for the PEF-assisted cellular uptake of the three topological forms of DNA, under a wide range of experimental conditions. In Fig. 2 *A*, cellular uptake of DNA (TR) is plotted against the concentration of $MgCl_2$ when 3T3 cells/DNA mixtures were treated with a single PEF of strength 2.5 kV/cm and of duration 5 ms. No differences were discernible for the four selected plasmid DNA, namely the sc-DNA, cr-DNA, and ln-DNA of $pRSV_{cat}$, sc-DNA of $pRSV_{neo}$, and $pRSV_{gpt}$. When the concentration of $MgCl_2$ was fixed at 5 mM and the duration of a PEF at 5 ms, TR as a function of the applied field strength is shown in Fig. 2 *B* for the three topoisomeric forms of $pRSV_{cat}$. Again, within experimental uncertainty, no differences were detected. TR increased with the increasing field strength, with an inflection point around 2.2 kV/cm. TR leveled off for field strength greater than 4 kV/cm. In Fig. 2 *B*, percentage cell survival is also given for each sample. At 4 kV/cm, most cells did not survive the PEF treatment.

Transient expression of cat gene

Transient expression of the loaded cat gene was measured by the cat activity in the cell extracts 2–3 days after the PEF

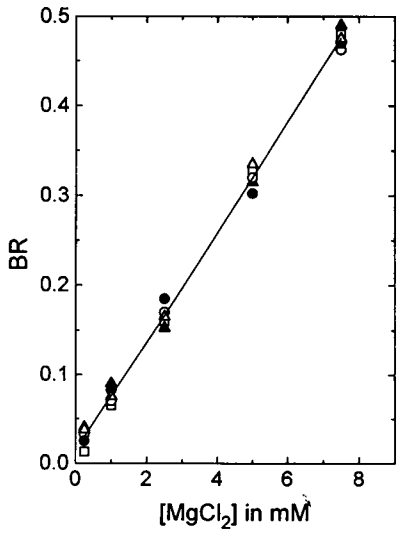

FIGURE 1 $MgCl_2$-dependent binding of DNA to NIH 3T3 cell surface. The binding ratio (CPM of [^{3}H]DNA bound to cell surface divided by total CPM) of sc-DNA (●), cr-DNA (○), and ln-DNA (△) of $pRSV_{cat}$, and sc-DNA of $pRSV_{neo}$ (□) and of $pRSV_{gpt}$ (▲) is plotted against $MgCl_2$ concentration. Each sample (500 μl) contained 5×10^6/ml cells and 0.1 μg of [^{3}H]DNA.

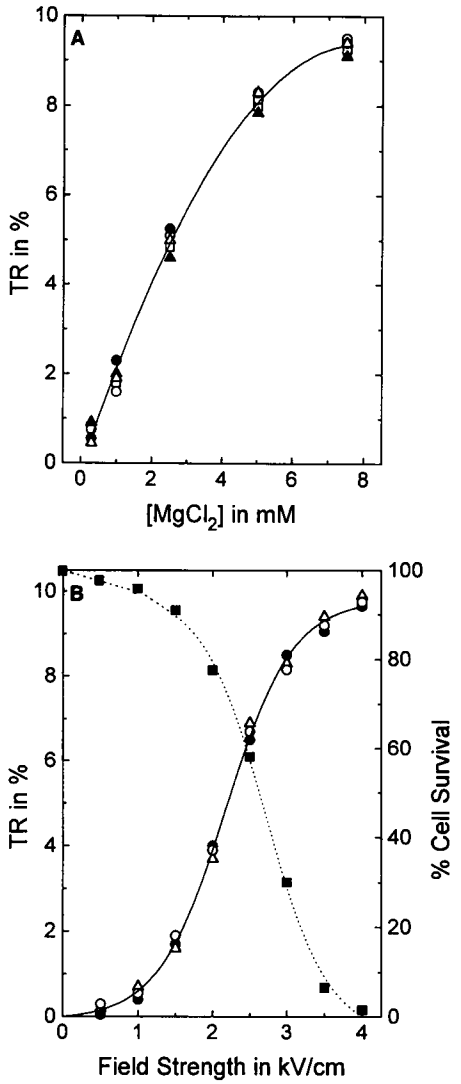

FIGURE 2 PEF-induced DNA uptake by NIH 3T3 cells. (*A*) Effects of $MgCl_2$: Each sample (150 μl) containing 5×10^6/ml cells was added 0.5 μg of [^{3}H]DNA and vortexed for 15 s. After incubation for 5 min, a single PEF of 2.5 kV/cm and of 5-ms duration was applied. After the procedure described in the Methods and Methods, DNA transfer ratio (TR, the CPM of [^{3}H]DNA loaded into cells divided by the total CPM) is plotted against the concentration of $MgCl_2$. Symbols are the same as in Fig. 1. (*B*) Dependence on the electric field strength: a single PEF of 5 ms with varied field strength was applied. The $MgCl_2$ concentration was 5 mM. Symbols are the same as in Fig. 1, except for the data on cell survival which are represented by ■.

treatment. Logarithm of the AR (acetylation ratio), as defined previously, was plotted against the concentration of $MgCl_2$ in Fig. 3 *A* for the three topoisomeric forms of $pRSV_{cat}$ when the cell/DNA mixture was treated with a single PEF of 2.5-kV/cm field strength and of 5-ms duration. In the entire range of $MgCl_2$ concentration studied, the AR was three to five times higher for the sc-DNA and cr-DNA compared with the AR for the ln-DNA. The AR for the sc-DNA and cr-DNA were identical within the experimental uncertainty. When the concentration of $MgCl_2$ was fixed at 4 mM and the pulse width of the PEF at 5 ms, the AR was dependent on the field strength of the PEF, as shown in Fig. 3 *B*. Here again the AR was consistently lower for the ln-DNA than for the sc-DNA and cr-DNA. The AR increased with increasing field strength and reached a maximum around 2.5 kV/cm. As was generally found in other studies, maximal transfection occurred around the point where 50% of the cells were killed by the PEF (Chang et al., 1992; Foster and Neumann, 1989). The autoradiograph of the thin layer chromatography of a typical experiment is shown in Fig. 3 *C*. Spots which migrated the slowest and stayed closest to the origin correspond to the unmodified chloramphenicol. Because there are two independent sites for acetylation, two mono-acetylated spots are seen in each sample (Kingston and Sheen, 1990).

Permanent expression of the PEF-loaded gene

Transient expression of the cat activity rose and peaked around 2–3 days after the transfection. This activity faded after few days. If the cat gene is not integrated into the host chromosomes, the gene vanishes and will no longer be expressed. Permanent expression of the loaded gene would indicate its integration into the host chromosomes. Fig. 4 presents permanent expression of neo gene in 3T3 for the three topoisomeric forms of DNA derived from $pRSV_{neo}$ and $pRSV_{gpt}$. Again, sc-DNA and cr-DNA show a much higher level of integration than the ln-DNA.

DISCUSSION

For the three cell lines we have investigated, namely, *E. coli JM*105, NIH 3T3 fibroblast, and HeLa cells, the $MgCl_2$-dependent binding of DNA to the cell surface and the PEF-assisted DNA entry into cells do not depend on the topology of DNA. Our experiments show that at given DNA concentration and $MgCl_2$ concentration, and with an identical PEF treatment, the amount of DNA loaded into the PEF-treated cells is proportional to the amount of DNA bound to the cell surface (comparing data in Fig. 1 with those in Fig. 2 *A*), irrespective of the DNA topology. These results are also consistent with our previous finding that binding of DNA to the cell surface is a leading event for the electrotransfection of cells. DNA which floats in the bulk has little incident for aligning itself to translocate the PEF-modified membrane sites, either by diffusion or by electrophoresis. The fact that PEF-assisted DNA entry does not depend on the topology of DNA would support the idea that the primary force of a PEF is the electrophoresis. The interaction energy of an electric field with a molecule depends on the net charge of the molecule. Electrophoresis of a molecule depends on the charge to mass ratio, which, at a given $MgCl_2$ concentration, should be the same for different topoisomers of DNA. Binding of $MgCl_2$ to different topoisomers of DNA could be different. But, at the concentration range we used in our experiment (up to 8 mM), such effects if at all present should be of secondary importance. $MgCl_2$ may also shield the charges in a DNA molecule, thus, reducing the electrophoretic driving force. Our experiments suggest that within the range of $MgCl_2$ concentration used, the $MgCl_2$-facilitated surface binding of

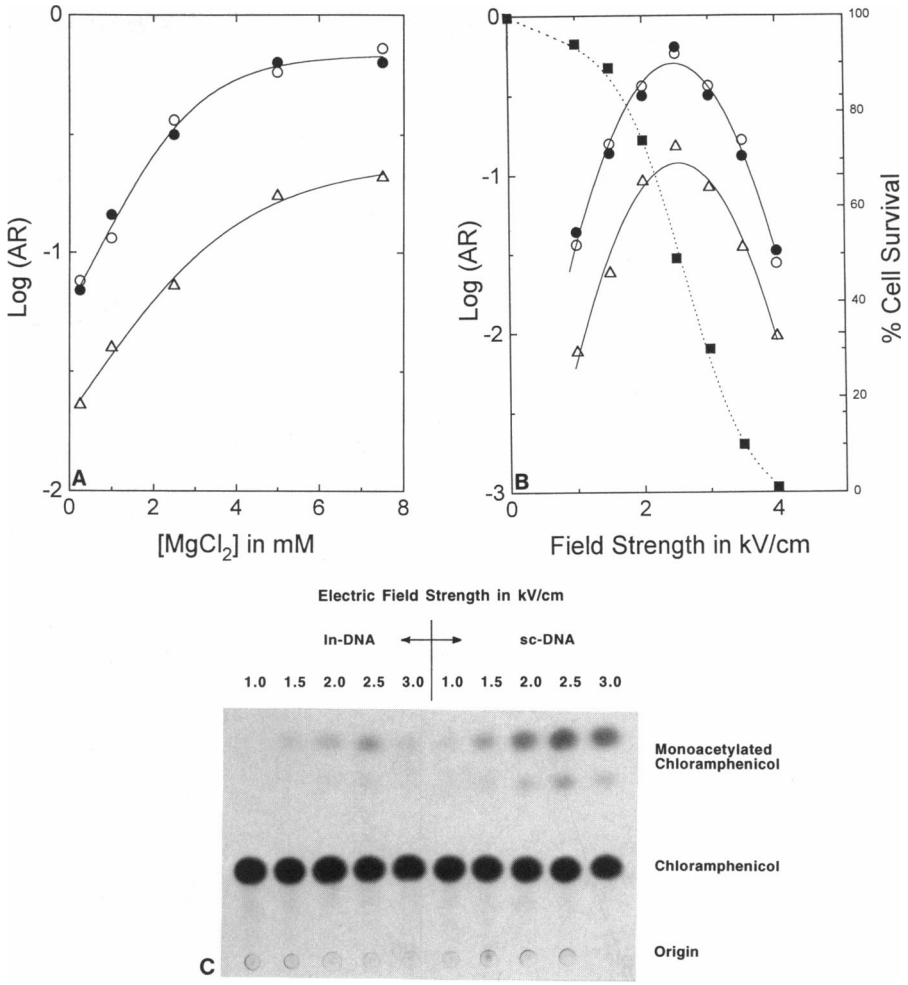

FIGURE 3 Transient expression of CAT activity after electrotransfection. (*A*) Effects of $MgCl_2$: NIH 3T3 (density at 1×10^7/ml) was mixed with DNA (2 μ/ml) and incubated on ice for 5 min. A single PEF of 5 ms to 2.5 kV/cm was applied. After 48–72 h of cell growth in the Complete Medium, cells were harvested. 20% of cell extract was assayed for cat activity as described in Material and Methods. Data are for sc-DNA (●), cr-DNA (○), and ln-DNA (△) of $pRSV_{cat}$. (*B*) Dependence on electric field strength: a single PEF of 5-ms duration and of varied field strengths was applied. $MgCl_2$ concentration was 5 mM. Other conditions and symbols are the same as in *A*. Data for the cell survival are represented by ■. (*C*) Transient expression of cat in transfected NIH 3T3 cells: autoradiographs of silica gel, which indicates the cat activity of the cell extracts from NIH 3T3 cells 3 days after the electrotransfection with $pRSV_{cat}$, are shown. Lanes 1–5 are samples transfected with the ln-DNA, and lanes 6–10 are samples transfected with the sc-DNA of $pRSV_{cat}$. A single PEF of 5-ms duration and of varied field strength was applied to the 3T3/DNA mixture. Experimental conditions are the same as in *B*. Increasing field strength of the PEF up to 2.5 kV/cm resulted in higher expression of the cat. Cells transfected with the sc-DNA show expression of considerably higher cat activity than cells transfected with the ln-DNA.

DNA is more important than the charge shielding effects. Although, the migration speed of DNA in a medium is inversely proportional to the friction and hence the shape of the molecule, for surface bound DNA to translocate across a cell membrane in a very short period of time, the membrane translocation process is unlikely to be friction-dependent.

DNA uptake by electro-osmosis flow (Dimitrov and Sowers, 1990) or endocytosis of cell membranes (Chernomordik et al., 1990) are also plausible. However, experiments by Klenchin et al. (1991) and Sukharev et al. (1992) have shown that the transfection efficiency was much higher if the polarity of a PEF was to drive the DNA toward the cells via electrophoresis than if the polarity was to drive DNA in the direction of electro-osmosis toward cells. This observation suggests that under their experimental conditions, electrophoresis dominates. However, if a high intensity PEF of long duration is used, electro-osmosis could become an important mechanism. Cellular uptake of DNA by the endocytosis of cell membranes was not likely because ln-DNA loaded into *E. coli* was rapidly degraded by the host enzyme (Xie et al., 1992). DNA enclosed in lipid vesicles was not accessible to binding by ethidium bromide (Chernomordik et al., 1990). It is inconceivable that DNA enclosed in a lipid vesicle would be accessible for rapid degradation by the endonucleases of the host cell.

In general, the efficiency of electrotransfection increases with increasing field strength, but the cell survival decreases with increasing field strength. The optimal conditions for achieving high TE usually occur at where 50% of cells perish (Chang et al., 1992). Data in Fig. 3 *B* and Fig. 4 agree with these observations. However, we have found that the maximal TE in our experiments occurred around 2.5 kV/cm for a 5-ms PEF. This is much higher than those reported by Tekle et al. (1991). They have found that the optimal TE occurred at 1.2 kV/cm with a PEF of 0.4 ms. The difference may be due to the fact that their experiments were done with cells in the monolayer culture and ours were done with cells in suspension. Most electrotransfection experiments are performed in media of low ionic strength in order to avoid severe Joule heating (Chang et al., 1992). Thus, our study of effects of $MgCl_2$ was limited to less than 10 mM. If an experiment has to be done at a higher salt concentration, surface binding of DNA is usually higher at higher salt concentrations at least up to the concentration we have studied, i.e., around 0.1 M NaCl (Xie et al., 1992).

In conclusion, we have shown that the transfection efficiency (TE), which has been used to measure the effectiveness of the PEF-assisted DNA uptake by cells, depends on many factors, some of which are not relevant to the study of mechanisms of electrotransfection, such as DNA stabil-

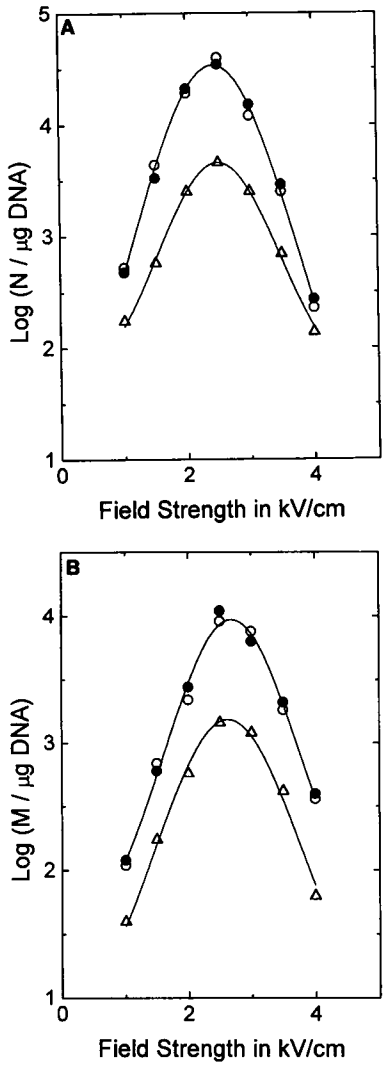

FIGURE 4 Permanent expression of neo and gpt genes in the transfected NIH 3T3 cells. (*A*) Dependence of $pRSV_{neo}$ transfection efficiency on electric field strength. The procedure for the identification of cells in which neo gene was expressed in the host chromosomes, is given in the Material and Methods. *N* represents the number of colonies. The cell/DNA mixture was treated with a PEF of 2.5 kV/cm and of varied field strength. $MgCl_2$ concentration was 5 mM. Data in ● are for transfection by the sc-DNA, in ○ are by the cr-DNA, and in △ are by the ln-DNA. *N* represents the number of colonies per 5×10^6 cells. (*B*) Dependence of $pRSV_{gpt}$ transfection efficiency on electric field strength. Experimental conditions and symbols are the same as in *A*. *M* represents the number of colonies per 1×10^7 cells.

ity in the host cell, the ability of DNA to express, and to integrate into the host chromosomes, etc. We have shown that, PEF-induced translocation of DNA across the membrane does not depend on the topology of DNA. However, the stability and the ability of DNA to express and to integrate into host chromosomes do depend on the topology of DNA. We have suggested that DNA translocation across the plasma membrane is driven by the electrophoretic motive force, and that only DNA bound to the cell surface is able to enter cells effectively.

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