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Short communication

729—ELECTRIC GENE TRANSFER INTO CULTURE CELLS *

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SUMMARY

The electric field method of gene transfer via electroporation of cell membranes is gaining importance in cellular and molecular biology. In particular the electroporation technique provides the low copy number transfection required for genetic studies. As demonstrated with mouse lyoma cells, with lymphoid cell lines and with plant protoplast, the electric method has appreciable advantages compared to biochemical procedures of cell transformation. Electroporation also provides a physically plausible mechanism for cell fusion in electric pulses.

The electric field method of gene transfer [1] is becoming increasingly important in cellular and molecular biology. In contrast to biochemical procedures like the calcium phosphate coprecipitation technique and the polyethylene glycol bound techniques, the electroporation method provides low copy number transfection [2]. A low number of foreign gene copies in the host DNA is a prerequisite for detailed molecular biological studies.

The electroporation technique of gene transfer into culture cells is based on the observation that cell membranes become transiently permeable by short electric impulses above a threshold field strength [2–6]. The electrically induced permeability increase leads to a transient exchange of matter across the perturbed membrane structure: partial release of soluble cytoplasma constituents and intrusion of solvent components into the cell interior. Electric impulses not only induce, or enhance, material exchange through membranes but they also cause membrane–membrane fusion when two membranes are in close contact with each other [7–9]. The application of short electric impulses to aggregated single cells of the microorganism *Dictyostelium discoideum* leads to the formation of giant cells (Fig. 1) with up to 50 nuclei, which as a whole develop, participate in the natural aggregation cycle, and are chemotactically active [8].

In contrast to statements made in Refs. 10 and 11, the cell fusion induced in

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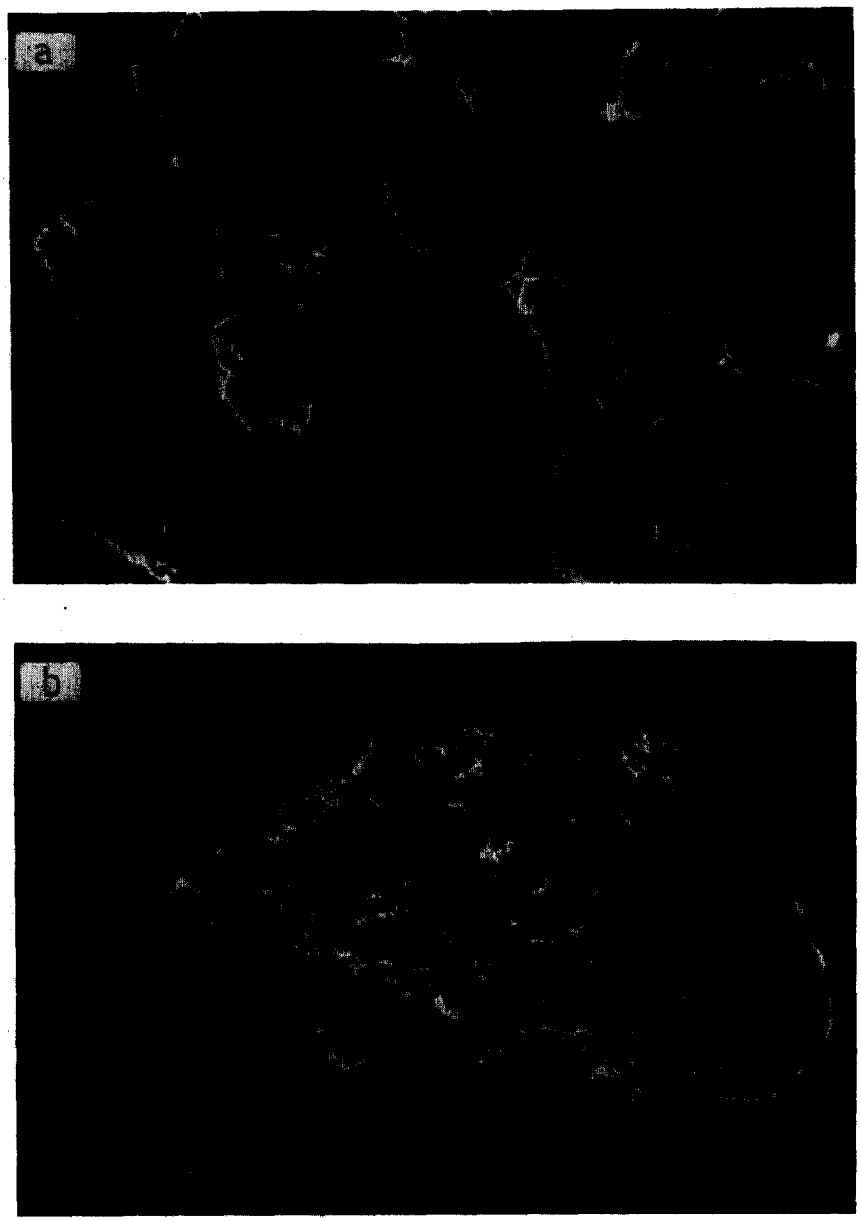

Fig. 1. Electrofusion of cells of the eukaryotic microorganism *Dictyostelium discoideum*. (a) Individual cells of the strain Ax-2. (b) Giant syncythia produced by electric impulses (6 kV cm^{-1}, 40 μs); see Ref. 8.

homogeneous electric fields does not require any chemical helper such as polyethylene glycol or any chemical manipulation of the cells. This field method is thus the simplest reported so far.

A further aspect of electric field effects on the membrane structure is the artificial transfer of macromolecules or larger particles into a cell interior [12]. An application of the electric pulse technique to genetic material was reported by Auer et al. [13]: uptake of SV 40 DNA and mammalian cell RNA into human red blood cells.

Recently, actual gene expression of electrically induced gene transfection in culture cells was demonstrated [1]. When an aqueous solution of circular or linear DNA carrying the thymidine kinase gene and of mutant mouse cells (LTK^- cells) deficient of this gene is subjected to a sequence of about three electric field pulses at 20 °C then, within a period of about 10 min after pulsing, the LKT^- cells take up large amounts of the plasmid DNA. The newly acquired thymidine kinase activity is demonstrated by the survival of the transformed cells in a selection culture medium. The first electric field experiments with LTK^- cells and DNA containing the TK gene showed that there is enhanced colony formation after pulsing compared to the non-pulsed cells [14]. However, compared to the fact that the homogeneous electric field simultaneously affects all cells in between the planar electrodes of the measuring cell, the number of stable transformants in the previous experiments was rather low. It was then recognized [1] that the pulsed cell suspension requires a time interval of about 10 min before it can be processed further. This time period is probably necessary to permit DNA penetration into the cell interior. There are a threshold of about 6–7 kV/cm and an optimum field strength range of $8(\pm 0.5)$ kV/cm for the DNA transfer leading to colony formation in the HAT selection medium. At higher field strengths the cells are irreversibly damaged [14,15].

Although the presence of divalent ions such as Mg^{2+} increases the amount of DNA bound to the cell surfaces [12], Mg^{2+} actually reduces and finally prevents gene transfer [1].

Simple incubation of LTK^- cells with DNA occasionally leads to the formation of a few transformants. Therefore, DNA adsorbed on the cell surface is able to penetrate the cell interior; the yield of colonies, however, is rather low compared to the colony yield after electric pulsing.

The detailed optimum conditions of the electric gene transfer depend on the cell type and the size of the cells and must be explored for every case along the lines described [1].

Among the more recent applications of the homogeneous electric field technique in gene transfer studies is the culture cell expression of immunoglobulin κ-genes [2]. By comparison of a fibroblast and a lymphoid cell system it was found that it is the initiation of the κ-gene transcription that is tissue specific. Correctly initiated transcripts of mouse immunoglobulin κ-genes were obtained when plasmids were introduced into the lymphoid cell line X 63 Ag.8.653. In contrast, gene transfer into the monkey kidney fibroblast cell line CV 1 resulted in incorrect 5′-termini. The plasmid DNA used was rearranged mouse Ig κ-genes with upstream regions of 90, 160, 370 and 870 base pairs, respectively, inserted into an SV 40 expression vector.

There is no activation of the κ-gene promoter in the fibroblast system by the SV 40 enhancer, irrespective of whether the functionally necessary upstream sequences are present or not.

Molecular biological investigations of this type require that the number of gene copies inserted into the host DNA is low. In contrast to classical biochemical techniques the electric-field-mediated transfection has the advantage of low copy numbers. The impulse method using homogeneous, exponentially decaying electric fields [2] is thus not only very simple and very efficient but also appears to be the method to choose to study gene expression at the physiological low dosage level.

At present the mechanisms of the electrically induced permeability change in membranes and of the penetration of cell surface adsorbed DNA into the cell interior are not known. For sure, the presence of an external electric field favours charge and dipole configurations which have a larger overall polarization in the field direction. This, in turn, may lead to a thinning of membrane patches, to hole formation, and finally, at high electric field strengths, to membrane rupture or dielectric breakdown.

The primary targets for electric-field-induced structural changes are probably the partially water-filled defect sites in the lipid bilayer organization. These defect sites may grow to pores of short life time. A general treatment of pore formation and of changes in the pore size induced by variations in pressure, temperature or electric fields is straightforward [1]. A specific model for electroporation and electrofusion phenomena of membranes has recently been developed [16].

In the framework of the electroporation model, structural transitions are described in terms of a period block organization. In the periodic block model, a block represents a nearest-neighbour pair of lipid molecules in either of two states:

(i) the polar head group in the bilayer plane; or

(ii) facing the centre of a pore.

The number of blocks in the pore wall is the stochastic variable of the model describing pore size and stability. The Helmholtz free energy function characterizing the transition probabilities of the various pore states contains the surface energies of the pore wall and the planar bilayer and, if an electric field is present, also a dielectric polarization term. Assuming a Poisson process, the average number of blocks in a pore wall is given by the solution of a non-linear differential equation. At subcritical field strengths, the pore radius increases and, on reaching a critical pore size, the membrane ruptures (dielectric breakdown). If, however, the electric field is switched off before the critical pore radius is reached, the pore apparently completely reseals to the closed bilayer configuration (reversible electroporation).

Gene transfer into cells requires previous adsorption of the DNA onto the membrane surface. Within the lifetime of a pore region nearby the adsorbed DNA, at least part of the DNA must penetrate the pore. It appears that in the annealing phase of the reversible electroporation process the entire DNA molecule crosses the perturbed membrane structure.

Clearly, the physicochemical analysis of electric-field-induced pores in lipid membranes is still rather unsatisfactory, representing at present only an attempt

towards understanding electroporation (and electrofusion) operative in electric gene transfection of culture cells. Since a simple version of the originally rather sophisticated electric field apparatus [3] is now commercially available (Neumann-Electroporator, Fa. Dialog, Harffstr. 34, D-4000 Düsseldorf, F.R.G.), the electroporation method of cell transfection can be used in any biological laboratory.

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