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High-velocity mechanical DNA transfer of the chloramphenicolacetyl transferase gene into rodent liver, kidney and mammary gland cells in organ explants and in vivo

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Mouse and rat liver, kidney and mammary gland explants were bombarded with high-velocity microprojectiles carrying a chloramphenicolacetyl transferase gene under different promoters (pTAT-cat, pχ-Casein-cat, pβ-Casein-cat). The expression of a CAT gene was revealed in all organ explants 24 h after transfection. The most pronounced expression was found when a TAT-CAT construction was used. In experiments in vivo rat liver was bombarded in situ with microprojectiles carrying pTAT-cat DNA. A marked activity of the CAT gene was detected 24 h after the bombardment.

Transfection; High-velocity microprojectile; CAT gene; Rodent cell in vitro and in vivo

1. INTRODUCTION

'Gene therapy' (gene replacement) based on the introduction of necessary foreign genetic information into genetically defective somatic cells is becoming more and more popular now among current approaches to the therapy of human genetic diseases (for review see [1]. Some work in this field is based on the transformation of somatic cells in vitro [2–6]. The authors of other publications have tried to introduce foreign genetic information directly into the cells of a whole animal organism using either retroviral constructions [10] or DNA injection into animal cells and organs in vivo [7–9].

In the present work, we applied to the direct gene delivery into the cells of animal organs in vitro and in situ an experimental approach based on biolistic methodology. The method consists of bombardment of the cells with microprojectiles (tungsten particles) covered with DNA containing the necessary genes. It was proposed and successfully used for the transfection and genetic transformation of plant cells in calluses and leaves. Delivery of foreign DNA into these cells by other methods of transfection was impossible because they had thick walls [10,11].

We have previously modified the method for the transfection of animal cells and demonstrated its applicability to the stable genetic transformation of cultured NIH 3T3 mouse cells [12].

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2. MATERIALS AND METHODS

2.1. *Transfection in vitro*

Pieces of mouse and rat liver, kidney and mammary glands, 1.5 × 1.5 mm in size, were placed in plastic Petri dishes with sheets of a Millipore filter on the bottom. The 199 culture medium supplemented with 10% of foetal calf serum (10%), insulin (5 μg/ml), dexametasone (5 μg/ml) and prolactine (5 μg/ml) was used. The explants were cultivated at 37°C in 5% CO_2 for 60–90 min, the excess of the medium was then removed, the explants were bombarded by tungsten microprojectiles with plasmid DNA, again cultivated for 20–24 h, and then taken for a CAT assay performed using a standard technique [13].

2.2. *Transfection in vivo*

Inbred rats of the Wistar line (2–3 months old) were anesthetized with ether and their abdomen cavity was opened. The liver was taken out and raised a little by means of sterile pads. The animal body, except part of the liver (3 × 3 cm) to be bombarded, was shielded from microprojectiles with a plastic screen. The distance between the end of the barrel and the liver surface was about 15–20 cm. Immediately after the bombardment, the liver was placed back into the abdomen cavity and the abdominal wall and skin were stitched up. The animals were sacrificed 24 h later, the bombarded area of the liver was removed and taken for a CAT assay [13].

2.3. *Bombardment technique*

The shooting technology was mainly similar to that used previously for the transfection of animal cells [12] and plant calluses [14]. The distance between the end of the barrel and the tissue surface was about 10–15 cm in experiments with organ explants and 15–20 with the liver in vivo. To reduce the contamination of cells with toxic products of the powder, two diaphragms with the center apertures of an increasing diameter were placed between the barrel and the bombarded tissue. The first diaphragm contained a metal grid 1 mm × 1 mm mesh in size and the second diaphragm had a grid 0.14 mm × 0.14 mm mesh in size. The first grid was located near the end of the barrel to decrease cell damage by the parts of a destroyed macroprojectile. The second grid was located as close as possible to the bombarbed tissues

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Fig. 1. Results of an acetylated chloramphenicol assay in organ explants 24 h after the bombardment of the tissues with microprojectiles carrying a CAT gene under different promoters. (a) mammary gland; (b) liver; (c) kidney; (1) CAT gene under a γ-casein promoter; (2) CAT gene under a TAT promoter; (3) CAT gene under a β-casein promoter. The most pronounced expression of the CAT gene can be seen in the liver tissue under a pTAT promoter.

and disintegrated the conglomerates of tungsten particles formed in the course of DNA precipitation. The use of these grids increased the efficiency of the transformation.

Particles of metal tungsten used in the bombardment had a diameter from 0.4 to 1.8 nm.

The following plasmids were used for transfection: pTAT-CAT, pγ-casein-CAT, and pβ-casein-CAT.

Plasmid DNA was prepared by standard lysozyme/alkaline lysis followed by CsCl gradient centrifugation. Calcium-phosphate precipitation [12] was used to coat the particles with plasmid DNA. 10 μl of a DNA solution (1 mg/ml) added to 10–15 mg of tungsten particles were taken per one shot.

3. RESULTS AND DISCUSSION

Our work was divided into two parts. In the first series of experiments mouse and rat liver, kidney and mammary gland explants taken in sterile conditions were cultivated for 60–90 min at 37°C and then bombarded with tungsten microprojectiles covered with CAT plasmid DNA under TAT, χ- and β-casein promoters. The bombarded explants were incubated for 24 h and after that were taken for a standard CAT assay.

Four separate shooting experiments were performed. The results of an acetylated chloramphenicol assay are presented in Fig. 1. The most pronounced expression of a CAT gene can be seen in the liver tissue under a TAT promoter. Another 3 experiments gave similar results.

Fig. 2 shows the results of an acetylated chloramphenicol assay in mouse mammary gland explants after shooting with a CAT gene under different promoters. Just as in the previous experiment, the most pronounced expression of the CAT gene can be seen when a construction under a TAT promoter was used.

In the second series of experiments, an attempt was made to introduce foreign genes directly into the cells of an organ in situ, i.e. as part of a whole organism. Rat abdomen was opened and the surface of the liver was bombarded with microprojectiles carrying plasmid DNA (pTATcat). After the bombardment, the wound was stitched up. The animals were sacrificed 20–30 h after the procedure. The CAT assay revealed a marked activity of chloramphenicol acetyl transferase in all bombarded livers (Fig. 3).

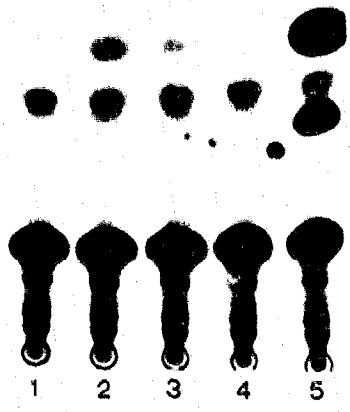

Fig. 2. Results of an acetylated chloramphenicol assay in a mouse mammary gland explant 24 h after the shooting with a CAT gene under different promoters. (1) negative control; (2) CAT gene under a TAT promoter; (3) CAT gene under a β-casein promoter; (4) CAT gene under a γ-casein promoter; (5) positive control. As in the previous experiments (Fig. 1) the most pronounced expression of the CAT gene can be seen when the construction with a TAT promoter was taken.

Our experiments have thus demonstrated that high-velocity microprojectiles can be used to introduce foreign genetic information into cells of rodent organs in explants and (in the case of liver) in situ, i.e. as part of a whole animal organism.

The results of high-velocity mechanical DNA injection of foreign genes into liver cells in situ are particularly noteworthy. Many plasmids were constructed specially for the expression in liver cells during recent years [2,3,5]. However, in fact, these genetic constructions were tested only in vitro, i.e. with cultured liver cells. Attempts to introduce these constructions into liver cells in vivo using biolistic technology seem to be reasonable. One may expect that stimulation of the regeneration process in the liver after its local damage

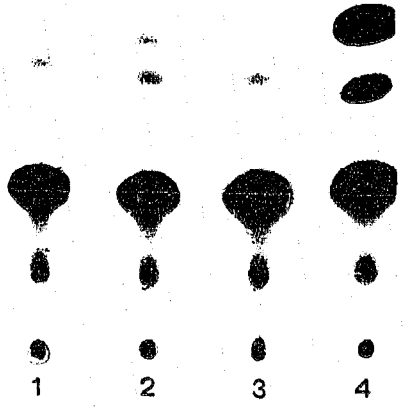

Fig. 3. Results of an acetylated chloramphenicol assay in rat liver in vivo 20 h after the bombardment with microprojectiles carrying a CAT gene under a TAT promoter. (1) experiment No 1; (2) experiment No 2; (3) negative control; (4) positive control.

by high velocity microprojectiles will make the introduced genes integrate into liver cells by homologous recombination [19] and cause their stable genetic transformation.

The possibility to use high velocity microprojectiles for foreign gene transfer into animal cells in vivo was suggested about two years ago by Sanford [11] and Zelenin et al. [12]. Henceforth some progress in this field was reported in the literature but only, at least to our knowledge, in the form of abstracts [15-17] or references to personal communications [18]. We give here a detailed description of such an investigation.

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