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Genetic transformation of mouse cultured cells with the help of high-velocity mechanical DNA injection

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NIH 3T3 mouse cells were transfected by the plasmid pSV3neo (G418-resistant) with the help of high-velocity mechanical DNA injection based on the principle of bombarding cells with tungsten particles covered with the DNA. Stable transformants were obtained. Dot-hybridization and Southern analysis revealed the integration into the genome of 5–20 copies per cell of original plasmid DNA. The plasmid DNA was shown to have tandem organization.

Transfection; High-velocity mechanical DNA injection; Antibiotic resistance; (NIH 3T3 cell)

1. INTRODUCTION

Gene transfer to animal cells plays a major role in molecular and cellular biology. Foreign genes can be introduced into cells with the aid of several standard methods (review [1]). New methods of transfection have been used to transform animal cells: these include osmotic transfection [2], laser transfection [3], transfection by scrape and sonication loading [4], strontium-phosphate transfection [5] and transfection by DNA-protein complexes]6].

Recently, it has been reported that plant cells can be transfected by using a new method in which high-velocity microprojectiles deliver DNA into the nuclei of the cells [7]. Here we have used this method for gene transfer into cultured animal cells.

2. MATERIALS AND METHODS

2.1. *Materials*

NIH 3T3 mouse cells [8] were used as a recipient to test the possibility of transfection. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and gentamycin (40 μg/ml) in 5% CO_2 at 37°C.

The plasmid pSV3neo [9], containing the G418-resistance gene, was used for transfection. Plasmid DNA was prepared by the lysozyme-NaOH procedure [10] followed by CsCl equilibrium density gradient centrifugation.

2.2. *Transfection of cells*

Exponentially growing cells were trypsinized, seeded at 1×10^5 cells per 5 cm plate and incubated overnight in 5 ml growth medium. 4.5 ml of the medium was then removed and cells were bombarded with high-velocity tungsten microprojectiles (0.1–1.0 μm diameter) coated with plasmid DNA. A special particle-gun similar to that described in [11] was used. Target cells were placed approx. 15–20 cm from the end of the device. Our protocol differed from the original [11] in that we used standard calcium-phosphate precipitation [12] instead of spermidine to coat the tungsten particles with plasmid DNA. 10 mg tungsten particles in 1 ml were used, 20 μg plasmid supercoiled DNA being used for one shot.

Immediately after the shot, the plates were placed in a CO_2 incubator (5%) for 15 min to restore membrane lesions and growth medium was subsequently added. On the next day the medium was removed, the cells were rinsed with fresh medium and divided into portions in the appropriate ratio (>1:5) on 10-cm plates and incubated for an additional 24 h before selection for stable transformants was begun in the presence of G418 (400 μg/ml, Sigma, USA). Cell viability was estimated after staining with fluorescein acetate (1 μg/ml) 24 h after bombardment.

To determine the frequency of stable transformation, cells were selected for 2–3 weeks, the growth medium being replaced every 3 days. The efficiency of transformation was calculated by dividing the average number of NEO^R colonies (>100 cells) by

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the total number of cells and approximated to the 1 μg plasmid DNA taken for the preparation of tungsten microprojectiles.

As a control, cells were bombarded with the tungsten microprojectiles without plasmid DNA or the calcium-phosphate DNA precipitate concentrated to 2 μl by centrifugation (13 000 × g, 30 min).

2.3. *DNA analysis*

Total DNA was isolated from 5×10^6 cells [13] and analysed by dot hybridization [14] or cleaved with *Eco*RI and blotted according to Southern [15] after electrophoresis. The radioactivity of the ^{32}P-labeled pUC18 probe, which was used in hybridization, was 2×10^8 cpm per μg plasmid DNA. After hybridization (65°C) in 6 × SSC, 5 × Denhardt, 0.5% SDS, 200 μg/ml salmon sperm DNA which was sonicated and melted, the filters were washed and radioautographed (−70°C).

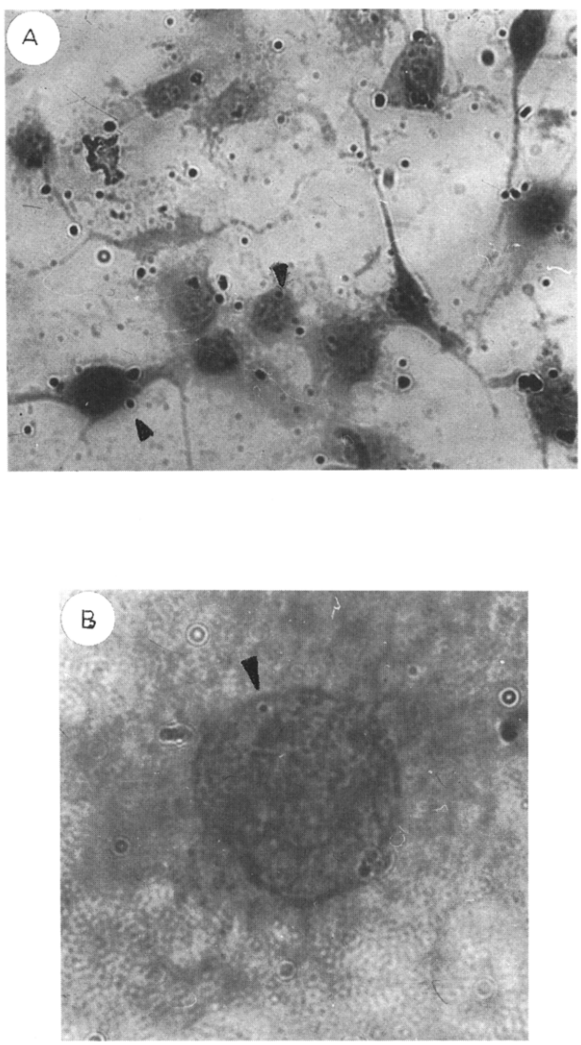

Fig.1. NIH 3T3 cells bombarded with tungsten microprojectiles (A); presence of particles in the cell nucleus (arrowheads) (B). Phase contrast.

3. RESULTS AND DISCUSSION

NIH 3T3 cells were bombarded by tungsten microprojectiles with the DNA of pSV3neo plasmid on their surface. Fig.1 demonstrates that shot particles penetrated the cells and are localized in the nuclei of some. As a result of 3 experiments, colonies of stable transformants were obtained (fig.2). The frequency of stable transformation was 1.1×10^{-5}/cell per μg plasmid DNA. Viability of cells was 88% (95% for controls). In control plates no colonies were seen. To prove that the colonies of stable transformants had a selective advantage due to plasmid DNA incorporation, they were separated using steel cylinders and cultivated under selective conditions till they reached 5×10^6 cells. Total DNA was then prepared. The results of dot hybridization of DNA of 5 individual colonies are shown in fig.3. All contain the plasmid DNA. Densitometric analysis revealed 5–20 copies of plasmid DNA per cell.

To characterize the state of plasmid DNA in the cells we analyzed the total DNA of two clones by digestion with *Eco*RI, which cleaved the original (pSV3neo) plasmid at one site, and subsequently

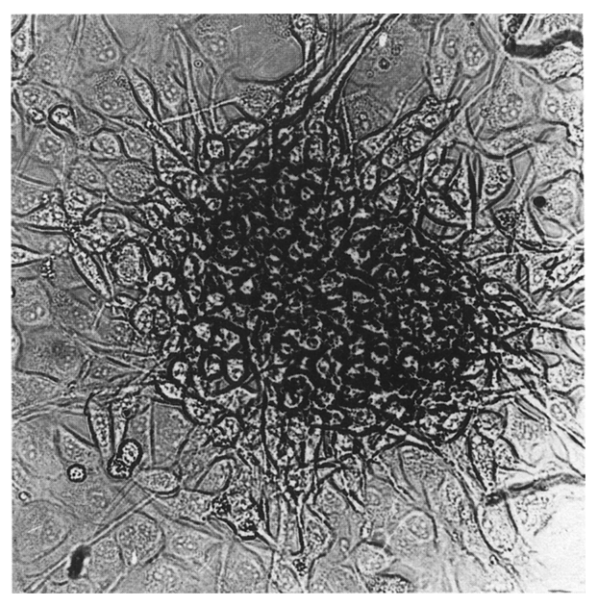

Fig.2. A G418-resistant clone on the 9th day of selection.

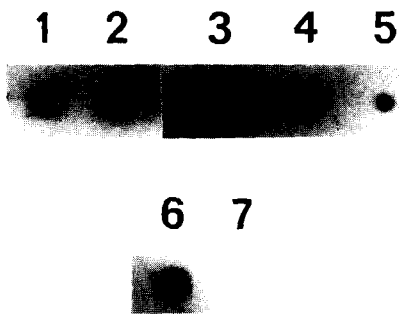

Fig.3. Dot hybridization of total DNA isolated from stable transformants (2 μg DNA per dot) with ^{32}P-labeled probe (pUC18). Dots: (1-5) DNA from transformants 1-5; (6) pSV3neo plasmid DNA corresponding to 70 copies per diploid genome; (7) DNA from NIH 3T3 cells.

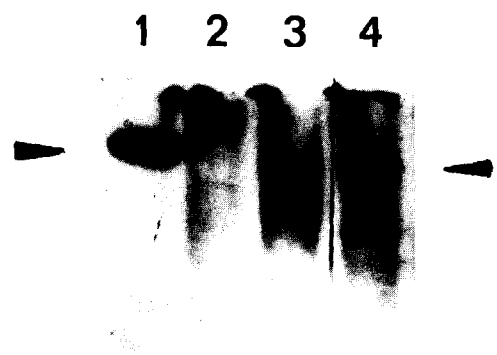

Fig.4. Southern blot analysis of DNA of stable transformants treated with *Eco*RI and separated by horizontal gel electrophoresis in 1% agarose (Sigma). After blotting the filter was hybridized with ^{32}P-labeled probe (pUC18). Lanes: (1) pSV3neo DNA digested by *Eco*RI corresponding to 60 copies per diploid genome, arrows; (2) DNA of NIH 3T3 cells treated with *Eco*RI; (3) DNA of clone 1; (4) DNA of clone 4. Lanes 2-4 contain 5 μg DNA.

electrophoresis. The results of blot hybridization are given in fig.4. The presence of bands with the same mobility as that of the original plasmid confirms that the clones originate from incorporation of plasmid DNA into the genome of the cells. The presence of additional bands with mobility which differs from that of the control can be explained by plasmid DNA rearrangement at the site of integration or by the existence of several sites of integration. Occurrence of a major band after hydrolysis by *Eco*RI can indicate tandem organization of plasmid DNA at the site of integration. The possibility of self-replication of pSV3neo plasmid in mouse cells can be excluded [12].

Our results indicate that the clones of stable transformants arose after bombardment of NIH 3T3 cells with tungsten particles covered with the pSV3neo plasmid DNA. The input of foreign particles into the cells' nuclei did not dramatically influence cell viability. Thus, the method of high-velocity mechanical DNA injection used originally for plant cells can be applied in the genetic transformation of cultured animal cells. The efficiency of this method is no greater than that of standard procedures but can have some advantages over other methods. Among such advantages the possibility of immediate DNA incorporation into the nucleus of the cell should be mentioned. The method can also be used for cells which are difficult or impossible to transfect by other methods.

REFERENCES

[1] Gordon, G.W. and Ruddle, F.H. (1985) Gene 33, 121-136.
[2] Lieber, M.R., Hesse, J.E., Nickol, J.M. and Felsenfeld, G. (1987) J. Cell Biol. 105, 1055-1066.
[3] Tao, W., Wilkinson, J., Standbridge, E.J. and Berus, M.W. (1987) Proc. Natl. Acad. Sci. USA 84, 4180.
[4] Fechheimer, M., Boylan, J.F., Parker, S., Sisken, J.E. and Patal, G.L. (1987) Proc. Natl. Acad. Sci. USA 84, 8463-8467.
[5] Brash, D.E., Reddel, R.R., Quanrud, M., Yang, K., Farrell, M.P. and Harris, C.C. (1987) Mol. Cell. Biol. 7, 2031-2034.
[6] Wienhues, U., Hosokava, K., Hoveler, A., Siegmann, B. and Doerfler, W. (1987) DNA 6, 81-89.
[7] De la Pena, A., Lorz, H. and Schell, J. (1987) Nature 325, 274-276.
[8] Jainchill, J.L., Aronson, S.A. and Todaro, G.J. (1969) J. Virol. 4, 549-553.
[9] Southern, P.J. and Berg, P. (1982) J. Mol. Appl. Genet. 1, 327-341.
[10] Birnboim, H.C. and Doly, J. (1979) Nucleic Acids Res. 7, 1513-1523.
[11] Klein, T.M., Wolf, E.D., Wu, R. and Sanford, J.C. (1987) Nature 327, 70-73.
[12] Graham, F.L. and Van der Eb, A.J. (1973) Virology 54, 538-539.
[13] Blin, N. and Stafford, D.W. (1976) Nucleic Acids Res. 3, 2303-2308.
[14] Kafatos, F.C., Jonec, C.W. and Efstradiadis, A.E. (1979) Nucleic Acids Res. 7, 1541-1552.
[15] Southern, E.M. (1975) J. Mol. Biol. 98, 503-507.
[16] Maniatis, T., Jeffrey, A. and Kleid, D.G. (1975) Proc. Natl. Acad. Sci. USA 72, 1184-1188.