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In vivo and *in vitro* gene transfer to mammalian somatic cells by particle bombardment

(transfection/transduction/gold)

NING-SUN YANG*, JOE BURKHOLDER, BETH ROBERTS, BRIAN MARTINELL, AND DENNIS MCCABE

Department of Mammalian Genetics, Agracetus, 8520 University Green, Middleton, WI 53562

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ABSTRACT **Chimeric chloramphenicol acetyltransferase and β-galactosidase marker genes were coated onto fine gold particles and used to bombard a variety of mammalian tissues and cells. Transient expression of the genes was obtained in liver, skin, and muscle tissues of rat and mouse bombarded *in vivo*. Similar results were obtained with freshly isolated ductal segments of rat and human mammary glands and primary cultures derived from these explants. Gene transfer and transient expression were also observed in eight human cell culture lines, including cells of epithelial, endothelial, fibroblast, and lymphocyte origin. Using CHO and MCF-7 cell cultures as models, we obtained stable gene transfer at frequencies of 1.7 × 10^{-3} and 6 × 10^{-4}, respectively. The particle bombardment technology thus provides a useful means to transfer foreign genes into a variety of mammalian somatic cell systems. The method is applicable to tissues *in vivo* as well as to isolated cells in culture and has proven effective with all cell or tissue types tested thus far. This technology may therefore prove to be applicable in various aspects of gene therapy.**

Direct transfer of biologically active foreign genes into mammalian somatic tissues or organs *in vivo* is an attractive strategy for gene therapy and the delivery of therapeutic proteins and peptides (1, 2). A number of methods have been reported to achieve this goal with varying degrees of success. These include the direct injection of naked plasmid DNA into muscles (2), delivery of DNA complexed with specific protein carriers (3), coprecipitation of DNA with calcium phosphate (4), encapsulation of DNA in various forms of liposomes (5, 6), and *in vivo* infection using cloned retroviral vectors (7, 8). We previously developed particle bombardment technology to accomplish genetic transformation of crop plants (9–14). In this report, we assess the possibility of direct *in vivo* gene transfer into mammalian somatic tissues by using this developing technology.

We have previously described the design of a high-voltage electric discharge device to be used for the acceleration of DNA-coated gold particles into living cells (10, 11). With this device a motive force is generated to accelerate the DNA-coated gold particles to high velocity, enabling efficient penetration of target organs, tissues, or single cells. We show that our particle bombardment technology can efficiently deliver foreign genes into liver, skin, and muscle tissues of rat or mouse *in vivo*. Expression of reporter genes in animals can be readily detected in the target organs at 1–2 days after gene transfer.

This technology can also be applied to solid tissue explants, organ slices, or organoids *in vitro*. Bombarded tissue materials can be monitored *in vitro* for specific experimental purposes or transplanted back into the body for gene therapy or other clinical purposes (1, 15). We show that the particle-bombardment method can effect gene transfer in freshly isolated mammary gland ductal segments and their derived primary cultures, as well as in various human cell lines. In the latter case, stable gene transfer was demonstrated in two established cell culture lines at a high transfection frequency. Our results suggest that this gene transfer method is applicable to a variety of different mammalian somatic cell systems.

MATERIALS AND METHODS

Particle Bombardment Device and DNA Delivery. The procedure for utilizing the particle bombardment device has been discussed in detail elsewhere (10) and involves several basic steps. Plasmid DNA in solution is first precipitated onto gold beads, which are chemically inert in most, if not all, biological systems. The DNA-coated particles are then resuspended in ethanol and evenly deposited onto a thin Mylar film. The Mylar sheet is then placed adjacent to two closely spaced electrodes, where an electric arc, generated by a high-voltage discharge, provides the motive force. The force accelerates the DNA-coated gold particles to high velocity, enabling efficient penetration of target organs, tissues, or single cells. With our particle bombardment device we can finely tune and adjust the velocity and resulting distribution of gold particles in various target tissues by varying the discharge voltage (3–25 kV), gold bead density, and bead size. For this study, 1- to 3-μm gold beads at a density of 2 × 10^6 beads (0.1 mg) per cm^2 were routinely used, with the velocity of the beads adjusted by using different discharge voltages as indicated. Plasmid DNA samples were loaded onto beads at 1 μg of DNA per mg of beads, corresponding to 10,000 copies of a 5-kilobase (kb) DNA molecule per gold particle (1–3 μm).

Plasmids. Five plasmid DNA constructions, RSV-CAT, RSV-Neo, RSV-Lux, MMTV-β-Gal, and CMV-β-Gal, were used as reporter genes. The RSV-CAT gene construction contains the Rous sarcoma virus (RSV) long terminal repeat (LTR) promoter and the chloramphenicol acetyltransferase (CAT) coding sequence (16). RSV-Neo contains the RSV LTR promoter and the neomycin phosphotransferase II (NPT II) coding sequence (17). RSV-Lux contains the RSV LTR promoter and the firefly luciferase coding sequence (2). CMV-β-Gal contains the cytomegalovirus (CMV) immediate early promoter and *Escherichia coli* β-galactosidase (β-Gal) coding sequence (18). The MMTV-β-Gal contains an RSV LTR enhancer, a mouse mammary tumor virus (MMTV) promoter (19, 20), and the β-galactosidase coding sequence. The MMTV-β-Gal expression vector was constructed by T. Mulcahy (University of Wisconsin, Madison) by inserting the *E. coli* β-galactosidase gene coding sequence into the pMAM-

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Abbreviations: RSV, Rous sarcoma virus; MMTV, mouse mammary tumor virus; CMV, cytomegalovirus; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase; NPT II, neomycin phosphotransferase II.

*To whom reprint requests should be addressed.

neo vector obtained from Clontech. The coding sequences for all of these reporter genes have a simian virus 40 polyadenylylation signal sequence at the 3′ terminus.

***In Vivo* Bombardment of Various Organs.** To perform *in situ* bombardment of liver tissues in live animals, we surgically opened the rat abdominal cavity and exposed the tip of a liver lobe as the target site. At energy settings of 10–14 kV bleeding was not visible to the naked eye. Small hemorrhage spots were detectable under low-magnification microscopy. The extent of these spots indicated tissue damage from blasting was minimal. After the *in vivo* bombardment the incisions were closed with sutures. After surgery the animals recovered from anesthesia in an hour and behaved normally. Forty-eight hours later the animals were sacrificed. The bombarded portion of liver lobe was excised, tissue was extracted, and CAT activity was assayed (21). For *in vivo* bombardment of mouse skin tissue, hair was removed from the abdomen by treatment with Nair (Carter-Wallace, New York) and the exposed epidermal tissue was bombarded at 10 kV. For bombarding mouse muscle tissue, abdominal muscle was surgically separated from the skin tissue layer, the exposed muscle was bombarded at 15 or 18 kV, and the animals were sutured closed. The mice were sacrificed 24–48 hr after blasting, and the bombarded organs were excised. Cell-free extracts were prepared and assayed for CAT activity.

***In Vitro* Bombardment of Tissue Cultures.** Ductal segments of human and rat mammary gland were isolated from excised mammary tissues by collagenase and hyaluronidase digestion of surrounding stroma tissue (22, 23). Mammary gland tissues and the derived cultures were generously provided by M. Gould (University of Wisconsin, Madison). MCF-7 cells, a human mammary carcinoma cell line, and other human cell lines were obtained from the American Type Culture Collection and were cultured under conditions recommended by that supplier (24). *In vitro* tissue samples, including mammary gland organoids, suspended cells, or monolayer cell cultures, were placed onto a Petri dish substratum and bombarded at appropriate voltage settings as indicated. Two days later, they were harvested from cultures and assayed for marker gene expression. When MMTV-β-Gal was used as a reporter gene for bombardment, target tissue or cell cultures were grown in corresponding culture media containing dexamethesone at 2 μg/ml, for induction of MMTV promoter activity (19).

Stable Transfection of MCF-7 and CHO Cells. MCF-7 and CHO (Chinese hamster ovary) cells were transfected by bombarding cells at 7 kV, 1 μg of RSV-Neo DNA per mg of beads, 0.1 mg of beads per cm^2. Bombarded cells were cultured for 1 day, transferred, and cultured for additional 2 days, and then grown under G418 (GIBCO) selection at 200 μg/ml. Antibiotic-resistant colonies appeared between 10 and 20 days after selection. A number of colonies were scored microscopically to obtain transfection frequency, and transfected cells were pooled by trypsinization. Stably transfected MCF-7 cells were cultured and subcloned for an additional 50 passages ($\approx$1 year) under G418 selection. CHO cell clones were cultured with G418 for 5–10 passages.

Tissue Extraction and Enzyme Assays. Bombarded liver, skin, muscle, and mammary tissues were extracted with CAT buffer (21) and centrifuged; aliquots of supernatant were assayed for CAT activity (21); and protein concentration was determined with the Bradford assay (Pierce). One-unit CAT activity standards (Pharmacia) were included in each assay; "% conversion" of [^{14}C]chloramphenicol to acetylated forms was measured and normalized to give total units of CAT activity. All assays were conducted within the linear range of the reaction. Specific activities were determined as unit of CAT enzyme per mg of total protein in tissue extracts. Histochemical staining of β-galactosidase activity in various test samples was performed as follows. *In vivo* bombarded skin and muscle tissues were excised from live animals and stained, without prior fixation, for β-galactosidase activity as described (25). Mammary gland organoids and their derived primary cultures and MCF-7 cells grown in tissue cultures were fixed with 0.5% glutaraldehyde in Dulbecco's phosphate-buffered saline, rinsed, and stained for β-galactosidase activity (25). Luciferase assays were performed with a Monolight 2001 luminometer (Analytical Luminescence Laboratories, San Diego) as described (2). Zymogram assays of NPT II activity in MCF-7 cell extracts were performed, within the linear range of the reaction, as previously described (26).

Southern Blot Analysis. Genomic DNA was purified according to Sambrook *et al.* (27). A 5-μg sample of transfected MCF-7 DNA was digested with the appropriate enzymes overnight and subjected to electrophoresis on a 0.7% agarose gel. The DNA was transferred to a nylon membrane and hybridized overnight to a 950-base ^{32}P-labeled NPT II riboprobe (28). Restriction endonuclease digestion of genomic DNA, transfer to nylon membranes, preparation of hybridization probes, membrane hybridization, and autoradiography were performed as previously described (9, 11, 27).

RESULTS AND DISCUSSION

To directly transfer functional genes to animal organs *in vivo*, we bombarded various target organs *in situ* in live animals. Fig. 1*A* and Table 1 show that *in vivo* expression of CAT activity was high for skin and liver tissues and lower but significant for muscle tissues. To obtain more quantitative data, normalizations were made for tissue thickness and protein concentrations in crude tissue extracts. Approximate specific activities were determined as units of enzyme per mg of protein as shown in Table 1.

The fraction of cells per target expressing the exogenous gene was determined by using a histochemical marker gene assayed at the cellular level. In this case, mouse skin and muscle tissues were bombarded with gold particles containing a CMV-β-Gal expression vector. Fig. 2*A* shows that about 20% of skin epidermal cells expressed high levels of β-galactosidase activity, demonstrating that the bombardment method is highly effective for *in vivo* gene transfer and expression in skin tissue. Fig. 2*B* shows the CMV-β-Gal

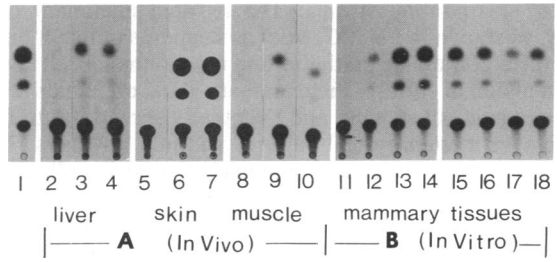

FIG. 1. Gene transfer and expression of RSV-CAT activities. (*A*) Rat liver, skin, and muscle tissues bombarded *in vivo*. Lanes: 1, activity standard of 1 unit of CAT; 2, liver (control—i.e., not bombarded); 3 and 4, liver bombarded at 10 and 14 kV, respectively, and assayed 48 hr after bombardment; 5, skin (control); 6 and 7, skin bombarded at 10 kV and assayed at 24 and 48 hr, respectively; 8, muscle (control); 9 and 10, muscle bombarded at 15 and 18 kV, respectively, and assayed at 48 hr. (*B*) Mammary gland tissues bombarded *in vitro*. Lanes: 11, rat mammary gland organoid (MGO) in 1-day-old culture, control (i.e., not bombarded); 12, 13, and 14, rat MGO bombarded at 3, 7, and 10 kV, respectively; 15, human MGO in 4-day-old culture, bombarded at 10 kV; 16, 10-day-old monolayer primary cultures derived from human MGO, bombarded at 10 kV; 17 and 18, rat MGO bombarded as freshly isolated ductal segments in suspension (12 kV). After 2 days in culture, the attached organoids (track 17) and the floating organoids (track 18) were assayed separately for CAT activity.

Table 1. Specific activity of RSV-CAT expressed in liver, skin, and muscle tissues bombarded *in vivo*

Tissue or standard	% conversion	CAT units/ mg protein
Liver 1	2.6	1.25
Liver 2	2.2	1.06
Liver control	0.21	0.09
1 unit CAT enzyme	37.6	—
Skin 1	25.6	8
Skin 2	38.4	17.5
Skin 3	19.2	5.2
Skin control	0.06	0.008
1 unit CAT enzyme	49.4	—
Muscle 1	3.0	0.7
Muscle 2	1.2	0.24
Muscle 3	1.9	0.34
Muscle control	0.05	0.014
1 unit CAT enzyme	52	—

CAT activity obtained from *in vivo* bombardment experiments shown in Fig. 1*A* was measured as "% conversion" to give total units of CAT activity. Specific activity was determined as CAT units/mg of protein. For liver, 5-mm-thick liver lobes were bombarded on both sides and extracted. As the gold beads penetrated to a depth of approximately 125 μm in this experiment (determined by histology; data not shown), a conversion factor for "tissue dilution," 125 μm $\times$ 2 (blasted on both sides)/5 mm = 1/20, was used to normalize the CAT specific activity for liver for purposes of comparison among different tissue types. Similarly, conversion factors of 1/6 and 1/1.6 were used to normalize specific activity for muscle and skin, respectively. Unbombarded controls gave background specific activities.

expression in muscle cells bombarded *in vivo*, where only 1–3% of the cells were found to express β-galactosidase activity. The reduced frequency in muscle is possibly due to interference during the bombardment process by the presence of the fascia on top of the muscle tissue. Subsequent experiments indicated that this interference can be reduced by employing larger gold particles and higher voltage (data not shown).

Ductal segments of human and rat mammary gland, also termed "mammary gland organoids" (22, 23), can be isolated from excised mammary gland tissue by enzymatic dissociation of surrounding stroma. The isolated mammary organoids, as shown in Fig. 2*C*, exist as segments of intact ductal structures containing epithelial and myoepithelial cell layers. When plated in culture, the mammary gland organoids attach to the plastic substratum and eventually spread out to form monolayer mammary cell cultures within a few days. They can also grow as organized mammary tissues in collagen gel cultures (22). Mammary gland organoid preparations and the primary cultures derived from them can be effectively implanted back into mammary or white fat pad tissues of recipient animals (23), thus providing an attractive approach for gene therapy.

We bombarded rat mammary organoids and primary cultures derived from these tissue explants at three different culture stages: (*i*) freshly isolated organoids that were bombarded prior to plating, (*ii*) 4-day-old primary cultures in which mammary organoids had attached to the plastic substratum and started to spread out as tissue aggregates in culture, and (*iii*) primary cultures in which mammary organoids had completely spread out and began to grow as monolayer cells. As shown in Fig. 1*B*, high RSV-CAT activities were detected 2 days after bombardment in all three sets of test samples. When primary cultures of human mammary epithelial cells were utilized in identical experiments, similar levels of CAT expression were observed (Fig. 1*B*, lanes 15 and 16). Using MMTV-β-Gal, we detected the expression of β-galactosidase in mammary gland organoids and derived epithelial cell cultures at the cellular level. In this case, dexamethasone (2 μg/ml) was added in culture media to induce MMTV promoter activity (19). Fig. 2 *C* and *D* shows that about 3–4% of bombarded cells (determined by cell counting under microscopy) exhibited β-galactosidase activity. Mammary cells present either as "intact" tissue explants in an organoid or as monolayer cells were capable of expressing β-galactosidase activity. Similar results ($\approx$3%) were also observed for β-galactosidase expression in MCF-7 cells, an established human mammary carcinoma cell line (Fig. 2*E*). A higher magnification, Fig. 2*E Insert*, shows the expression of β-galactosidase activity in two MCF-7 cells, each containing a single gold bead within the cell.

As shown in Fig. 2*A*, up to 20% of skin cells were found to exhibit CMV-β-Gal activity, while only 3–4% of mammary cells in organoid or cell cultures show MMTV-β-Gal activity. A comparative study of the CMV-β-Gal and MMTV-β-Gal genes was therefore conducted in MCF-7 cells. We have found that while only 3–4% of MCF-7 cells exhibit β-galactosidase activity in transfection with MMTV-β-Gal (as shown in Fig. 2*E*), approximately 25% of MCF-7 cells exhibit β-galactosidase activity when transfected with CMV-β-Gal (Fig. 2*F*). The CMV promoter is strong and highly constitutive (29). The MMTV promoter, on the other hand, is tissue specific and dexamethasone inducible (19). Results shown in Fig. 2 *E* and *F* suggest that the lower expression frequency observed with MMTV-β-Gal may underrepresent the number of effectively transfected cells due to the relative weakness of the MMTV promoter. Therefore, the particle bombardment method for gene transfer may also be useful for rapid assays of promoter strength or tissue specificity under both *in vivo* and *in vitro* conditions.

Enzymatic activities shown in Figs. 1 and 2 were obtained at 48 hr after bombardment and represent transient gene expression. These levels of activity were found to last an additional 2–7 days (4–9 days in total), depending on the organ or cell type tested. This period of transient gene expression is followed by a highly variable decline in activity over the following few days (data not shown). Additional experiments using the luciferase gene as the reporter showed that, among tissues tested, rat muscle had the slowest decline in transient activity. Luciferase enzyme activities in rat muscle measured at 1, 3, and 14 days after bombardment were 5525 $\pm$ 2575, 9557 $\pm$ 5849, and 5721 $\pm$ 1467, respectively (activity expressed as mean relative light units per sample, $\pm$SD, n = 3).

To determine whether the particle bombardment method could also transfer genes stably, we employed CHO and MCF-7 cell lines and a G418-selectable marker gene (pRSVNeo) (17), which confers antibiotic resistance to transfected cells. Stable transfections were obtained at frequencies of 1.7 $\times 10^{-3}$ (10 independently bombarded cultures) and 6 $\times 10^{-4}$ (n = 3) for CHO and MCF-7 cells, respectively. Considered together with our results from transient gene assays, those frequencies indicate that stably transfected cells are recovered from effectively transfected cells at a rate of approximately 10^{-2} (i.e., $\approx 1.7 \times 10^{-3}$ for 20%). With consideration of the plating efficiency, the stable transfection frequencies (1.7×10^{-3} to 6×10^{-4}) obtained with the particle bombardment method compare favorably with frequencies commonly obtained with other DNA-mediated gene transfer methods. These include the 10^{-4} to 10^{-5} rate observed for electroporation (30, 31), $\approx 10^{-5}$ for the calcium phosphate method (31), and 2 to 5 $\times 10^{-4}$ for the lipofectin method (32). CHO and MCF cells were between 85% and 97% viable at 24 hr following bombardment, based on trypan blue exclusion. We therefore suggest that particle bombardment is less or no more deleterious to cell viability than other transfection treatments. These results of stable transfection obtained from

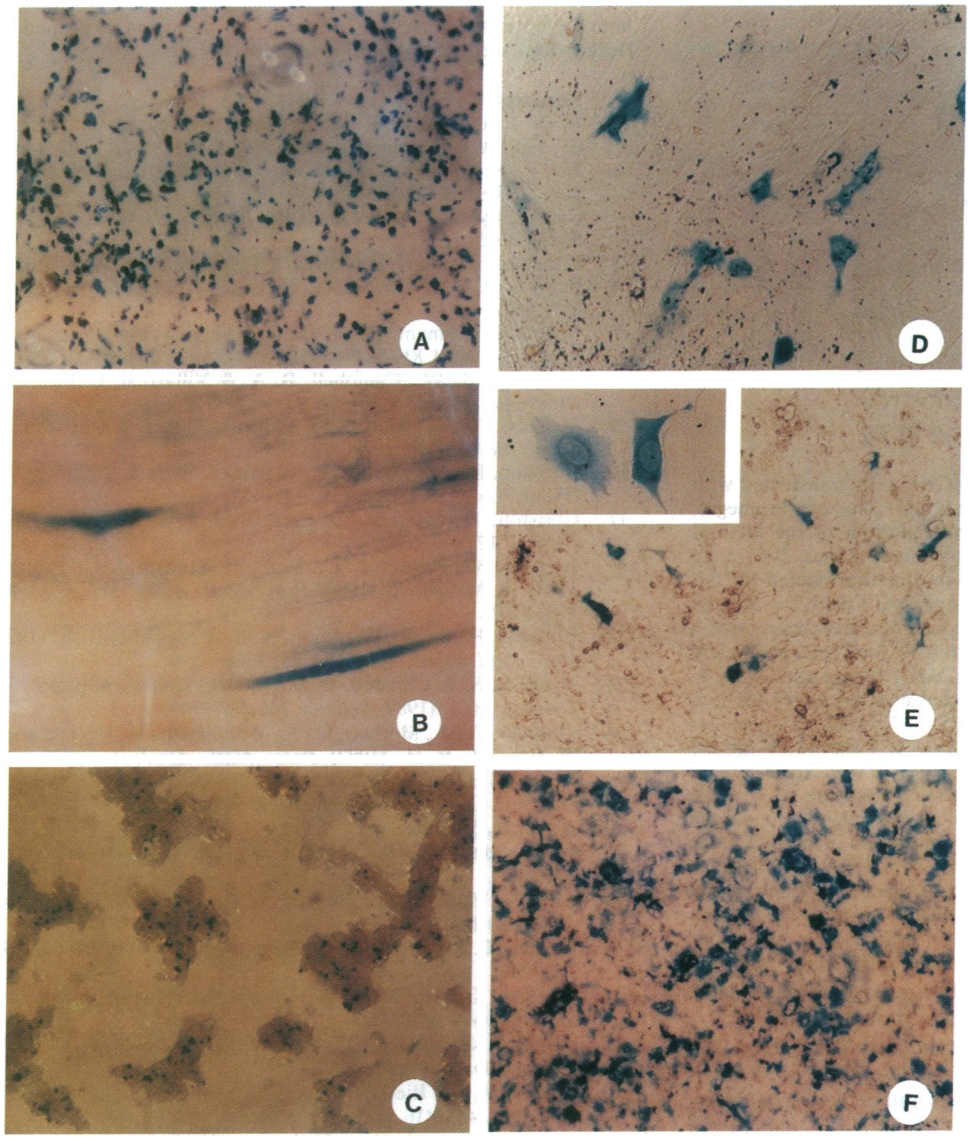

FIG. 2. Expression of CMV-β-Gal or MMTV-β-Gal activities observed at the cellular level for various blasted tissues. (*A*) Mouse skin tissues bombarded *in vivo* with CMV-β-Gal DNA. (*B*) Mouse muscle tissues bombarded *in vivo* with CMV-β-Gal DNA. (*C*) Mouse mammary gland organoids bombarded *in vitro* with MMTV-β-Gal DNA. (*D*) Primary cultures of human mammary epithelial cells bombarded with MMTV-β-Gal DNA. (*E*) Human mammary carcinoma cell line (MCF-7) cells bombarded with MMTV-β-Gal DNA; *Inset* at high magnification. (*F*) MCF-7 bombarded with CMV-β-Gal DNA. Mouse skin and muscle tissues were bombarded *in vivo* at 10 and 15 kV, respectively. Mammary gland organoids and their derived primary cultures and MCF-7 cells were bombarded at 10 kV. Forty-eight hours later, test tissues were excised from live animals or were harvested from tissue cultures and stained for β-galactosidase activity as described (25). The blue spots indicate the cells expressing β-galactosidase activity. (*A*, ×55; *B*, ×45; *C*, ×25; *D*, ×150; *E*, ×72; *E Inset*, ×375; *F*, ×72.)

in vitro experiments also suggest that if future *in vivo* experiments can be carried out with appropriate selection pressures, an appreciable starting population of stably transfected cells can be enriched or proliferated to increase long-term gene expression. Alternatively, functional genes constructed as episomal expression vectors may be used to confer long-term expression in target organs of live animals.

The G418-selected MCF-7 cell colonies were subsequently pooled, subcloned and propagated as mass cultures for more than 50 passages. Expression of the NPT II gene in stably transfected MCF-7 cells was detected by enzyme activity assays, and it was found not to be dependent upon the continued presence of G418 in the culture media (Fig. 3). Southern blot analysis (Fig. 4) demonstrated that the input NPT II gene is present in the stably transfected MCF-7 cell line at a copy number in excess of 20 per cell (compare lanes 3–6 and lane 11). In undigested DNA (Fig. 4, lane 10) the NPT II sequences comigrate with high molecular weight genomic DNA. Maintenance of the NPT II gene is associated with rearrangement of the pRSVNeo plasmid sequences (compare lanes 7 and 12), probably involving integration into the MCF-7 cell genome (33). Detailed analysis of the molecular organization of NPT II genes in transfected MCF-7 and CHO cells will be reported elsewhere.

In addition to MCF-7 and CHO cell lines, eight human cell culture lines, including cells of epithelial, endothelial, fibroblast, and lymphocyte origin, were tested for transient gene expression after particle bombardment. Variable, but readily detectable, RSV-CAT activities were obtained for each of these cell lines (data not shown).

Recently, Wolff *et al.* (2) demonstrated that plasmid DNA can be introduced into mouse muscle cells by direct injection of DNA into muscle tissue. An intriguing finding of their study is that months after DNA delivery, high levels of reporter gene expression were still detectable, while the introduced marker genes persisted as nonintegrated, circular plasmids that did not replicate. It will be interesting to

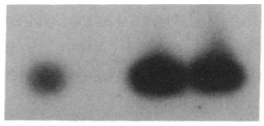

FIG. 3. Stable expression of NPT II activity in MCF-7 cells transfected with pRSVNeo plasmid DNA. Lanes: 1, *E. coli* NPT II enzyme activity standard (1 ng); 2, nontransfected MCF-7 cells; 3, transfected MCF-7 cells cultured under selection pressure (200 μg of G418 per ml); and 4, transfected MCF-7 cells that were cultured without selection pressure for 8 passages (2 months). Stably transfected MCF-7 cells were cultured for more than 50 passages under G418 selection (sample for lane 3). Before assaying for NPT II activity, a subline of these transfected MCF-7 cells was grown for 2 months in culture medium without G418 selection (sample for lane 4).

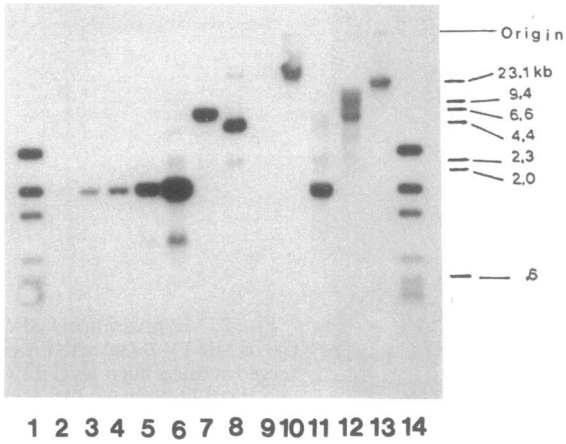

FIG. 4. Southern blot analysis of MCF-7 cells transfected with pRSVNeo. Lanes 1 and 14, 50 ng of pGEM DNA markers (Promega), which hybridize to the vector polylinker portion of the NPT II riboprobe. The fragment sizes are indicated. Lanes 2–6, copy number standards of pRSVNeo added into nontransfected MCF-7 DNA and double digested with *Bsu*36I and *Sma* I (lane 2 = 1 copy; lane 3 = 10 copies; lane 4 = 20 copies; lane 5 = 100 copies; lane 6 = 500 copies, based on a genome size of 6×10^9 base pairs per diploid cell). Lane 7, pRSVNeo digested with *Nde* I (single cut). Lane 8, undigested pRSVNeo. Lane 9, undigested nontransfected MCF-7. Lane 10, undigested transfected MCF-7 (T-MCF-7). Lane 11, T-MCF-7 digested with *Bsu*36I and *Sma* I. Lane 12, T-MCF-7 digested with *Nde* I. Lane 13, T-MCF-7 digested with *Kpn* I. *Bsu*36I and *Sma* I excise the intact NPT II coding sequence as a single fragment of 1.6 kb; *Nde* I cuts the pRSVNeo plasmid once and linearizes it; no restriction site for *Kpn* I is present in the pRSVNeo DNA. The blot was exposed to x-ray film for 24 hr with an intensifying screen. The 1-copy standard (lane 2), which is not detectable here, is apparent after exposure for 72 hr (data not shown).

determine whether this mode of stable gene transfer could also be demonstrated by using other means of direct gene transfer, including the particle bombardment method.

In summary, we have shown that delivery of foreign DNA to mammalian cells by means of particle bombardment is possible for a wide range of cell types and cell environments. The direct physical delivery of DNA into the nucleus or cytoplasm obviously bypasses specific barriers due to membrane receptors. Therefore, this method may serve as a general method for gene transfer into mammalian cells. It may also provide a useful alternative for the retrovirus-based vector systems. The method can be applied under *in vivo* conditions to liver, skin, and muscle, and presumably to other organ types. Many types of solid tissues or organs can now be considered as target tissues for direct gene transfer. This method can also be applied *in vitro* to various tissues, including surgically excised or biochemically dissociated organ segments or tissue clumps, as well as their derivative primary cultures. The particle bombardment procedure involves minimal manipulation of target organs, tissues, or cells and is versatile, efficient, and flexible. We therefore suggest this technology may prove to be applicable in various aspects of somatic cell gene therapy.

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