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Use of Electroporation To Introduce Biologically Active Foreign Genes into Primary Rat Hepatocytes

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A method is described for introducing and expressing cloned genes in isolated hepatocytes. Primary rat hepatocytes isolated by collagenase perfusion were transfected in suspension with plasmid pSV2CAT by electroporation. Forty-eight hours later, soluble extracts from transfected hepatocytes showed chloramphenicol acetyltransferase activity comparable to that obtained in rat hepatoma cell line H4AzC2 by calcium phosphate or DEAE-dextran transfection. The latter two methods could not be used successfully for primary hepatocytes because of cytotoxicity of these reagents. This indicates that electroporation is a useful method to obtain transient expression of foreign genes in primary epithelial cells, such as rat hepatocytes, which are difficult to maintain in cell culture.

During the past decade, a variety of methods have been developed for introducing foreign genes into eucaryotic cells, including calcium phosphate precipitation (9), DEAE-dextran (15, 22), protoplast fusion (18, 20), electroporation (16, 17), retrovirus and other viral vectors (3, 5), and liposomes (10, 19). Alternatively, DNA can be microinjected directly into the cell nucleus (1, 6), and by all these methods multiple copies of foreign genes can be introduced into individual cells. The efficiency of introduction and expression of foreign DNA by these methods is strongly dependent on the cell type used, and most studies have been conducted with immortalized or permanent cell lines (12). However, with primary cells in culture, successful transfection and expression of foreign DNA has been difficult to achieve (7).

In previous studies, we have used human hepatoma cell lines to study expression of introduced genes and the role of hepatitis B virus sequences as promoter-enhancer signals when engineered into plasmid expression vectors (R. Tur-Kaspa, R. D. Burk, Y. Shaul, and D. Shafritz, Proc. Natl. Acad. Sci. USA, in press). In an attempt to extend these studies to nontransformed liver cells, we found that primary rat hepatocytes cannot be transfected by the two most commonly used methods, calcium phosphate precipitation and DEAE-dextran. This is due to the inherent cytotoxicity of these transfection vehicles for primary hepatocytes. In the present study, we introduced DNA into isolated rat hepatocytes by electroporation (16, 17) and assayed recipient cells for expression of introduced foreign DNA with a plasmid containing the bacterial gene for chloramphenicol acetyltransferase (CAT), which is not normally present in eucaryotic cells (8).

The plasmid pSV2CAT is a specifically designed expression vector which contains the beta-lactamase gene and the origin of replication of pBR322 coupled to a simian virus 40 early transcriptional promoter-enhancer region driving the coding sequence for CAT (13). As a negative control, we used pA10CAT2, which is similar to pSV2CAT but lacks an effective enhancer element as a result of a deletion in the 72-base-pair repeat of the simian virus 40 fragment.

Sprague-Dawley rats (200 to 250 g), maintained under standard conditions, were the source of hepatocytes. Single-cell suspensions of hepatocytes were prepared by the procedure of Berry and Friend (2), with the collagenase perfusion mixture of Leffert et al. (14). Hepatocytes were cultured in RPMI-1640 (GIBCO Laboratories) supplemented with 100 U of penicillin per ml and 100 μg of streptomycin per ml (250 μg of amphotericin B [Fungizone] per ml was also included on the first day of culture). This medium was supplemented with a defined mixture of hormones, growth factors, and trace elements as reported by Enat et al. (4). Rat hepatoma cell line H4AzC2 was maintained in culture in Dulbecco modified Eagle medium (GIBCO Laboratories) plus 10% fetal calf serum supplemented with 100 U of penicillin per ml–100 μg of streptomycin per ml–250 U of amphotericin B per ml.

DNA was introduced into primary hepatocytes or rat hepatoma cells by transfection with calcium phosphate by the method of Graham and van der Eb (9), by transfection with DEAE-dextran by the method of Sompayrac and Danna (22), or by electroporation by the method of Potter et al. (17). For electroporation, plasmid DNA was first linearized by restriction enzyme digestion. Linearized plasmid vector (50 μg) was then added to 10^7 freshly prepared hepatocytes in 0.65 ml of sterile phosphate-buffered saline. The DNA and cells were incubated for 15 min at 0°C in a 1-ml disposable cuvette to which electrodes had been applied (17). An electric pulse was then delivered from a model 194 Isco power supply at a setting of 2.0 kV and the current at the maximum of 0.9mA. The current and wattage were set to a value of 5 on a scale of 100. The cells and DNA were allowed to sit for 15 min at 0°C, after which 10 ml of hormonally defined medium was added. Cell viability was determined by Trypan Blue dye exclusion, and the cells were replated. Twenty-four hours after plating, the medium was removed, and fresh medium was added. Forty-eight hours after transfection, cells were harvested, and extracts were prepared by sonication and centrifugation.

Expression of foreign genes in primary rat hepatocytes and rat hepatoma H4AzC2 cells transfected with plasmids pSV2CAT and pA10CAT was determined by assaying for CAT activity by the method of Gorman et al. (8). Transfection of hepatocytes with calcium phosphate or DEAE-

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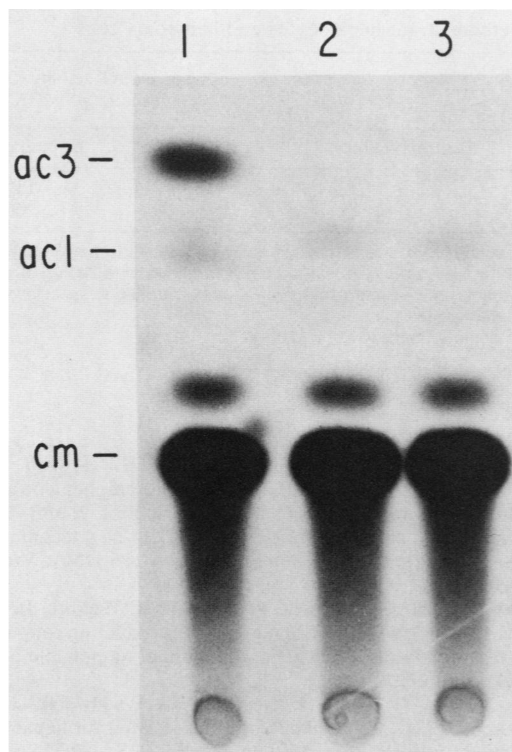

FIG. 1. Expression of CAT in primary hepatocytes transfected with pSV2 CAT by electroporation. DNA was introduced into primary rat hepatocytes by electroporation, as described by Potter et al. (7). Forty-eight hours after transfection, cells were harvested, and extracts were prepared by sonication and centrifugation. Cell extracts (40 μl; 200 μg of protein) were assayed for CAT activity essentially as described by Gorman et al. (8) with incubation for 1 h at 37°C. Twenty microliters of ethyl acetate-extracted samples were spotted on silica gel thin-layer plates, and [^{14}C]chloramphenicol (cm), 1-acetate chloramphenicol (ac1), and 3-acetate chloramphenicol (ac3) were detected by autoradiography. Lanes: 1, extract of cells transfected with pSV2CAT; 2, extract of cells transfected with pA10CAT2; 3, extract of mock-transfected cells.

dextran was ineffective, because cells died soon after transfection. Attempts to modify the respective protocols by reducing the amount of calcium phosphate or DEAE-dextran used, shortening the period of contact between hepatocytes and these agents, or addition of postincubation washing steps still resulted in cell death. In contrast, primary rat hepatocytes were successfully transfected by electroporation. All three transfection methods were successful with rat hepatoma cells. Figure 1 shows determinations of CAT activity in cell extracts of rat hepatocytes transfected by electroporation with pSV2CAT (lane 1) compared with cells transfected with control plasmid pA10CAT2 (lane 2) and mock-transfected cells (lane 3). The percent acetylation of ^{14}C-labeled chloramphenicol per 200 μg of protein in cell extracts was 2.3% with pSV2CAT, 0.02% with pA10CAT2, and 0.01% in nontransfected cells.

To compare transfection efficiency of calcium phosphate and electroporation, we transfected hepatoma H4AzC2 cells by both techniques. Figure 2 shows that significant CAT activity was detected in cell extracts of H4AzC2 cells transfected with pSV2CAT by using calcium phosphate or electroporation. Table 1 presents quantitation of the amount of CAT activity obtained with primary rat hepatocytes and rat hepatoma cells with calcium phosphate transfection versus electroporation. With electroporation, 10^7 cells were transfected, and cell survival was 50%, whereas with calcium phosphate precipitation, 10^6 cells were plated per 100-mm dish, and there was nearly 100% viability. Also, we used 50 μg of DNA per 10^7 cells in the electroporation method in contrast to 15 μg of DNA per 10^6 cells in calcium phosphate precipitation. There was no significant difference between the efficiencies of these transfection methods for H4AzC2 hepatoma cells. With primary hepatocytes transfected by electroporation, CAT activity was comparable to that observed in hepatoma cells transfected by calcium phosphate precipitation, i.e., 2.3% [^{14}C]chloramphenicol conversion to acetylated derivatives with hepatocytes versus 1.5% [^{14}C]chloramphenicol conversion with hepatoma cells. These results suggest that electroporation may represent a useful method for introducing genes into primary epithelial or other cells which are difficult to transfect or maintain in cell culture.

An important consideration in using various transfection methods is whether transient or stable transformation is desired. In the present study, our principal interest was in finding transfection conditions which would permit transient expression of foreign genes in primary hepatocytes. For this reason, we selected plasmid constructs containing an expressable gene (CAT) which is not present in eucaryotic cells and can be assayed within 24 to 48 h after introduction of DNA. This was important, because primary hepatocytes are difficult to maintain in long-term culture (4, 11). In addition, studies have shown that the level of CAT activity correlates with the level of CAT mRNA and thus provides a

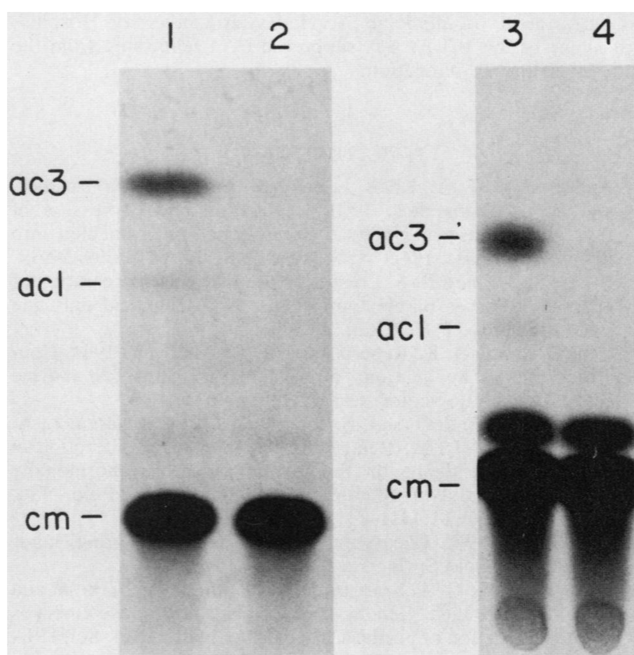

FIG. 2. Comparison of CAT activity in rat hepatoma H4AzC2 cells transfected with pSV2CAT or pA10CAT2 by the calcium phosphate versus the electroporation method. [^{14}C]chloramphenicol (cm) and its acetylated forms, 1-acetate chloramphenicol (ac1) and 3-acetate chloramphenicol (ac3), were detected by autoradiography. Lanes: 1 and 2, extracts of cells transfected by electroporation with pSV2CAT and pA10CAT2, respectively; 3 and 4, extracts of cells transfected by calcium phosphate precipitation with pSV2CAT and pA10CAT2, respectively.

TABLE 1. CAT activities produced by various DNA transfection methods in rat hepatocytes and hepatoma cells[a]

Cell type	% Chloramphenicol acetylation produced by:				Relative CAT activity (fold) produced by pSV2CAT[b]	
	pA10CAT2		pSV2CAT			
	CaP[c]	Electroporation	CaP[c]	Electroporation	CaP[c]	Electroporation
Rat hepatocytes		0.02		2.3		115
H4AzC2	0.01	0.01	1.5	1.6	150	160

[a] CAT activities produced by pA10CAT2 and pSV2CAT plasmids in primary rat hepatocytes and H4AzC2 cells transfected by calcium phosphate precipitation or electroporation were compared. Cells were harvested 48 h after transfection and analyzed for CAT activity (8) with 1 h of incubation with 200 μg of protein cell extract at 37°C. Percent acetylation of ^{14}C-labeled chloramphenicol was determined by thin-layer chromatography and liquid scintillation spectroscopy of acetylated and nonacetylated bands identified by autoradiography of the thin-layer chromatography plates.

[b] The values shown represent the fold increase in CAT activity produced by pSV2CAT over that obtained with pA10CAT2.

[c] CaP, Calcium phosphate precipitation.

measure of both transcription and expression of the introduced foreign gene (21).

By simple comparison, the electroporation method produced comparable results in primary hepatocytes and rat hepatoma cells, and results in hepatoma cells were similar by either calcium phosphate precipitation or electroporation. These studies indicate that electroporation of DNA into primary rat hepatocytes represents a viable method of introducing expressable foreign genes into nontransformed epithelial cells. Future studies will address the question of whether stable phenotypic transformation of hepatocytes can be obtained with this method.

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