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EFFICIENT LIPOFECTION OF HUMAN TROPHOBLAST CELLS IN PRIMARY CULTURES

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Human choriosomatomammotropic hormone, also known as placental lactogen, is expressed in syncytiotrophoblast cells of the placenta. Studying transcriptional regulation of the genes coding for this hormone, we became interested in transfecting primary cultures of these trophoblast cells. In this study, we show that it is possible to transfect, by the lipofection method, these giant cells in an efficient and reproductible manner. We show the presence of an enhancer region downstream from the hCS-B gene, functionally active in these cells ; furthemore, we demonstrate the placenta-specific characteristic of this enhancer, previously identified in a human choriocarcinoma cell line.

The study of the transcriptional regulation of a gene generally requires transfection of *in-vitro*-grown cells with various promoter/enhancer mutants linked to a reporter gene. Cells from primary cultures, although presenting closer-to-normal physiology and metabolism, are not often used in transient expression assays : in primary cultures, the cell yield is usually too small to sustain a large-scale transfection program (particularly in the case of human cells) and such cells are often hard to maintain in culture and to transfect.

In search for transcriptional regulatory elements involved in the expression of the human placental lactogen genes (choriosomatomammotropic hormone,hCS : 1, 2), we became interested in

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using primary cultur s of trophoblast cells from human placentas. These cells are easily obtained in large quantities. Mononuclear cytotrophoblasts are isolated from placental villi by enzymatic digestion and purification on Percoll gradient (3). In culture, cytotrophoblasts aggregate, fuse, and form syncytiotrophoblasts (Figure 1) which, about 48 hours after plating, secrete chorionic gonadotropin and placental lactogen (4). In order to introduce into these cells various plasmid constructs containing potentially regulatory regions of the hCS-B gene, we chose the lipofection technique (5) which, because of its low toxicity for the cells, can be well adapted to primary cultures.

MATERIALS AND METHODS

Cell isolation and culture. Term placentas were obtained after elective caesarean section from mothers near term with uncomplicated pregnancies. Villous tissue was dissected free of membranes and vessels, rinsed and minced in calcium and magnesium-free Hank's balanced salt solution and submitted to trypsine-DNase digestions as previously described (3, 4). The resultant cell suspension was carefully layered over a discontinuous Percoll gradient (5-70% in 5% steps) and

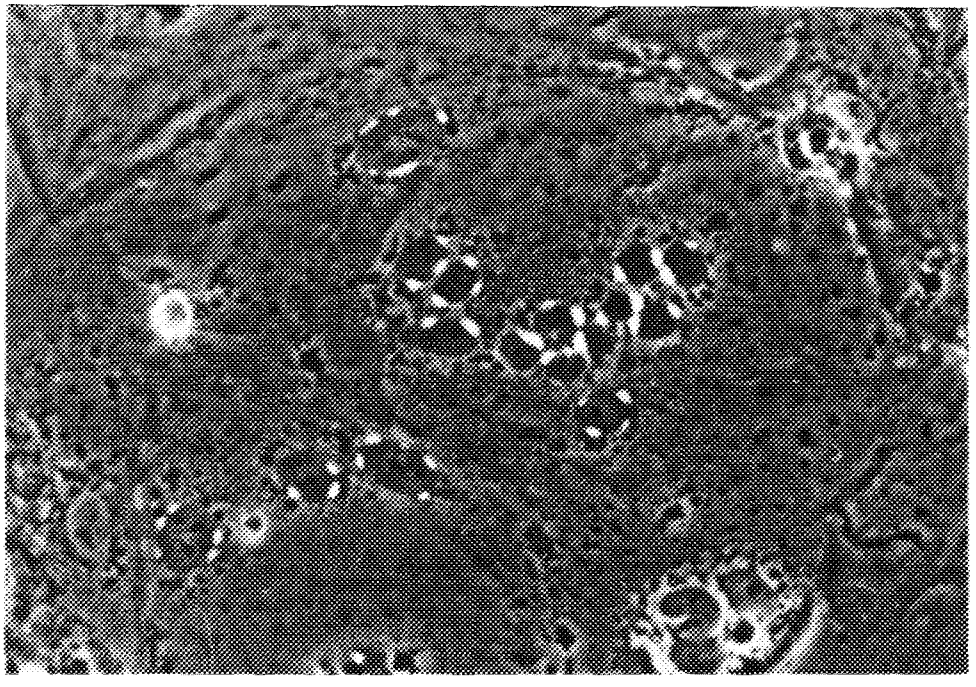

Figure 1. Morphology of trophoblastic cells in culture, showing syncytia formations.

centrifuged. After centrifugation, the homogeneous population of mononuclear cells contained in the middle layer (density, 1.048-1.062 g/ml) was isolated. This fraction consisted of more than 90% cytotrophoblast cells as previously reported (6). For primary culture, the cells were diluted to a concentration of 10^5 cells/ml with DMEM-H supplemented with 2mM glutamine, 20% heat inactivated fetal calf serum (FCS), and antibiotics: 100 µg/ml streptamycin, 100 IU/ml penicillin. The cells were plated in 60-mm Nunc culture dishes (3.10^6 cells in 3 ml) and incubated in humidified 5% CO_2-95% air, at 37°C. The medium was changed daily. Characterization of the cultured cells has been published elsewhere (6).

Lipofection and CAT assays. For lipofection, 15 µl of Lipofectin (Gibco-BRL) diluted to 100 µl with Opti-MEM (Gibco-BRL) was mixed with purified plasmid DNA (for the tested quantities, see below) and 2 µg of pRSV-ßgal (8), as an internal control, diluted to 100 µl with Opti-MEM. The DNA-liposome mixture was incubated for 10 min at room temperature and 1.3 ml of fresh medium supplemented with 10% FCS was added. Then, the old culture medium (on a 60-mm dish) was removed and replaced with the 1.5 ml of liposome mixture. After incubation for a variable time (see below) at 37°C, this medium was removed and replaced with 3 ml of FCS-supplemented fresh culture medium ; the cells were harvested by scraping. The cells, suspended in 200 µl of 100 mM Tris-HCl pH 7.6, were disrupted by three cycles of freezing/ thawing with sonication and the extracts briefly centrifuged to remove cell debris. Protein concentrations were determined (8) with bovine serum albumin as a standard. The CAT (chloramphenicol acetyl transferase) and ß-gal activities were then determined as described (9).

RESULTS AND DISCUSSION

Optimization of the lipofection conditions.

The lipofection technique is simple, reproducible and very efficient. It requires, however, the optimization of certain parameters. The optimal parameter values tend to vary according to the cell type. Below, we list the parameters that we found to be crucial for efficient lipofection. In parentheses, we give for each parameter the value range tested in our attempts to optimize the process for syncytiotrophoblasts :

1/ the presence or absence of serum during lipofection (we have observed that, according to the cell type, serum can have a positive or negative effect on transfection) ; 2/ the time spent by the cells in contact with the DNA-liposome complexes (1 to 24 hours) ; 3/ the amount of transfecting DNA (4 to 24 µg DNA per 60 mm dish) ; 4/ the time spent in cell culture prior to lipofection (1 to 3 days after plating) ; 5/ the time course of reporter gene expression (depends on the reporter gene : for the CAT gene, 24 to 72 hours).

In this experiment, the plasmid used was the pBLCAT2 vector (10), where the thymidine kinase promoter (TK) is linked to the CAT reporter gene.

We have determined the following optimal conditions promoting high CAT expression : mix 6 µg of plasmid DNA with 15 µl of Lipofectin (Gibco-BRL), incubate 10 min at room temperature, and add to 1.5 ml of DMEM with 10% heat inactivated, fetal calf serum. This suspension is layered over 3.10^6 cells having grown for 48-72 hours in 60 mm dishes. After 18 hours, the culture medium is changed and the cells are grown for a further 24 hours. The cells are harvested by scraping in 200 µl of 100 mM Tris-HCl pH 7.6, extracts are made and CAT activity is determined as described above. Under these conditions, high levels of CAT activity are assayed in the cell extracts.

The region downstream from the hCS-B gene acts as an enhancer in the syncytiotrophoblast cells.

Previously, an enhancer has been found 2 kb downstream from the hCS-B gene, using JEG-3 cells. This choriocarcinoma cell line, which does not express hCS, originates from cytotrophoblasts. In this work, we wanted to show the functional reality of this enhancer using the only cells expressing hCS, that is to say, syncytiotrophoblast cells.

The plasmids used were the pBLCAT2 vector and pCSB-CAT, where the downstream region of the hCS-B gene, corresponding to the sequences identified as enhancer in JEG-3 cells (11), has been introduced upstream from the hybrid TK-CAT gene of the pBLCAT2 plasmid.

Under optimized conditions for lipofection, the transcriptional activity developed by the pCSB-CAT plasmid is, on average, 25 times that displayed by the pBLCAT2 vector (Figure 2).

With this result, we show the placental specificity of the enhancer. It is interesting to note that this enhancer also seems to be active in cells derived from cytotrophoblasts although hCS is not expressed in these cells. To verify this interesting data using cells which are not derived from carcinoma, a time course of enhancer activation will be realized during the cyto-/syncytiotrophoblast differenciation using the primary culture system. Dissection of the enhancer region will also be carried out to precisely localize the important elements for its activity.

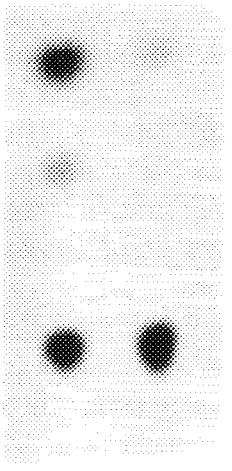

Figure 2. Activation of pCSB-CAT in syncytiotrophoblast cells ; 3.10^6 cells were transfected with 6 µg of plasmids complexed with 15 µl of Lipofectin. 1/ pBLCAT2 plasmid ; 2/ pCSB-CAT plasmid. CAT assays were performed as described in the text.

In conclusion, trophoblast cells from term placenta in culture are suitable for transfection by lipofection. This makes them a practical tool for molecular biologists studying genes expressed in the placenta, such as hormone, growth factor and growth factor receptor genes.

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