

Transfection of PC-12 cells: a model system for primary neuronal cells

Karen Kelly-Spratt

Center for Developmental Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA

A wide variety of mammalian cell types is used in gene transfer studies. Finding transfection reagents that enable highly efficient DNA uptake into a broad spectrum of cell types has become increasingly important. One cell line, PC-12, originally derived from a transplantable rat pheochromocytoma, is relatively difficult to transfect. PC-12 cells are small (5–10 μm), have a limited amount of cytoplasm, and have a long doubling time (>2 days). One important feature of PC-12 cells is their ability to respond to nerve growth factor (NGF), and therefore serve as a model system for primary neuronal cells. NGF-treated PC-12 cells cease proliferation, grow long neurites, and show changes in cellular composition associated with neuronal differentiation (1). Once PC-12 cells have become differentiated, the efficiency of transfection by many of the current methods such as calcium phosphate, lipofection, and electroporation, drops further. We investigated the efficiency of SuperFect™ Transfection Reagent, a proven reagent based on activated-dendrimer technology, to transiently transfect both undifferentiated and differentiated PC-12 cells.

Materials and methods

Undifferentiated adherent PC-12 cells were cultured in DMEM supplemented with 10% horse serum (HS) and 5% fetal bovine serum (FBS). Transfection of differentiated PC-12 cells required prior stimulation of adherent PC-12 cells with 50 ng/ml NGF (Regeneron Pharmaceuticals). NGF was supplied for a minimum of 5 days before transfection in either DMEM supplemented with 10% HS and 5% FBS, or in DMEM supplemented with 0.05% FBS (low-serum conditions). ▶

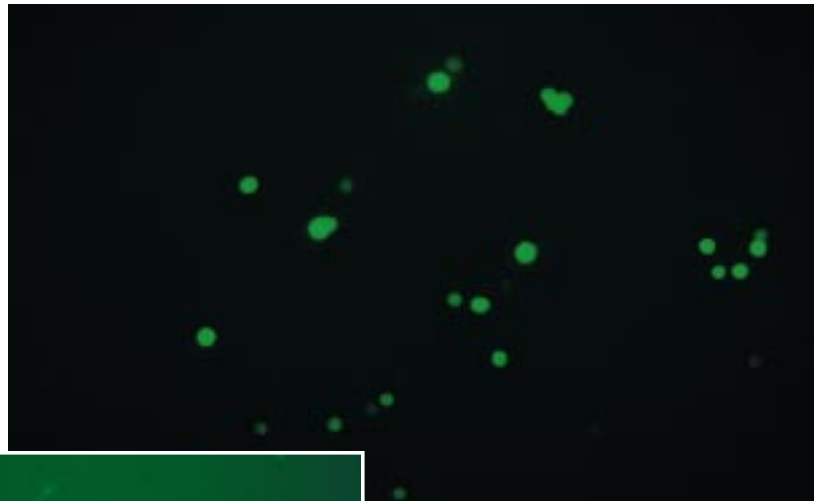


Figure 1 Expression of green fluorescent protein (GFP) from undifferentiated PC-12 cells 48 h post-transfection using SuperFect Reagent. 10^4 – 10^5 cells were plated per 60-mm dish one day prior to transfection. Transfections were performed in 2 ml growth medium (DMEM plus 10% HS and 5% FBS) using 3 μg pGFP (CLONTECH) and 15 μl SuperFect Reagent.

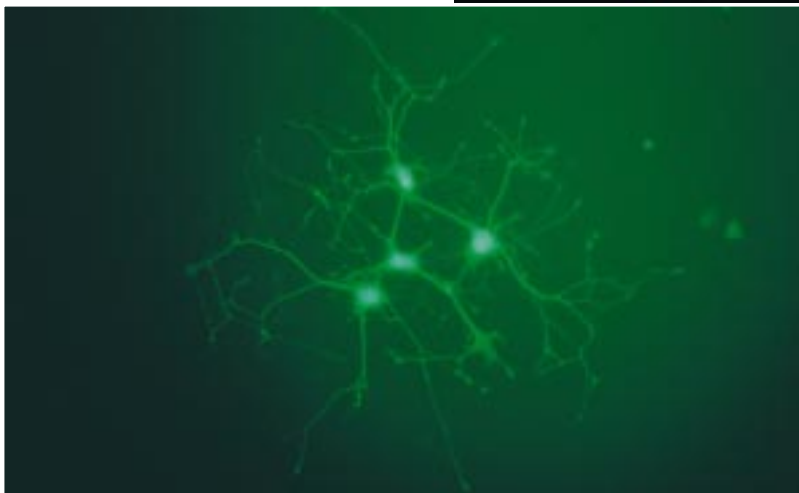


Figure 2 Expression of green fluorescent protein (GFP) from differentiated PC-12 cells 5 days post-transfection using SuperFect Reagent. 10^4 – 10^5 cells previously stimulated with 50 ng/ml NGF were plated per 60-mm dish one day prior to transfection. Transient transfections were performed in 2 ml low-serum growth medium (DMEM plus 0.05% FBS) using 3 μg pGFP (CLONTECH) and 15 μl SuperFect Reagent.

References

1. Greene, L.A. and Tischler, A.S. (1976) Establishment of a noradrenergic clonal cell line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2424.
2. Xia, Z., Dickens, M., Raingard, J., Davis, R., and Greenberg, M. (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* **270**, 1326.

Approximately 10^4 – 10^5 undifferentiated or differentiated cells were plated per 60-mm dish the day prior to transfection. Undifferentiated cells were transfected in 2 ml DMEM supplemented with 10% HS and 5% FBS, whereas differentiated cells were transfected in 2 ml DMEM supplemented with 0.05% FBS. Cells were transfected using a modified version of the green fluorescent protein (GFP) plasmid (CLONTECH). DNA–SuperFect complexes were formed using 3 µg or 5 µg pGFP and 5 µl SuperFect Reagent per µg plasmid DNA. After 2–3 h of incubation with the DNA–SuperFect complexes, the cells were washed with PBS and 5 ml fresh growth medium was added. Cells were evaluated for GFP expression 24, 48, and 72 h post-transfection by viewing under a fluorescence microscope. Each experiment was repeated at least 10 times.

Results

Within 12 h post-transfection of undifferentiated PC-12 cells, the first few green fluorescent cells were faintly visible. The intensity of fluorescence increased over time, and the overall transfection efficiency peaked at 25–30% after 48–72 h in the undifferentiated PC-12 cells (Figure 1). The NGF-treated differentiated PC-12 cells also displayed initial signs of GFP expression 12 h post-transfection. The fluorescence was mainly concentrated within the cell body over the next 48 h as the fluorescence intensity increased. When the cultures were left for

additional time intervals up to 7 days post-transfection, the GFP expression was seen throughout the entire cell, spreading out to the tips of the neurite extensions (Figure 2). Since differentiated cells cease to proliferate, GFP expression remained at least 10 days after transfection. The overall transfection efficiency was lower in the differentiated PC-12 cells (approximately 5%). However, this still represents a 2–3 fold higher efficiency than seen with other transfection methods as reported in the literature (2). No differences were found between the transfection efficiencies obtained for differentiated PC-12 cells grown in normal serum-containing medium and low-serum-medium conditions prior to transfection. 3 µg DNA gave slightly better transfection efficiencies than 5 µg DNA with both undifferentiated and differentiated PC-12 cells.

Conclusions

We have found SuperFect Transfection Reagent to be easy to use and to give higher transfection efficiencies for PC-12 cells than other widely used transfection reagents (2) or calcium phosphate. The ability of this activated-dendrimer reagent to deliver DNA into differentiated PC-12 cells is especially striking, since these cells are no longer mitotically active. In addition, the transfection efficiencies obtained using SuperFect Reagent are highly reproducible, as determined by comparing efficiencies from numerous replicate experiments. ■

Acknowledgement

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Ordering Information

Product	Contents	Cat. No.
SuperFect Transfection Reagent (1.2 ml)	For 40 transfections in 60-mm dishes or 160 transfections in 12-well plates	301305
SuperFect Transfection Reagent (4 x 1.2 ml)	For 160 transfections in 60-mm dishes or 640 transfections in 12-well plates	301307