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Efficient DNA transfection of quiescent mammalian cells using poly-L-ornithine

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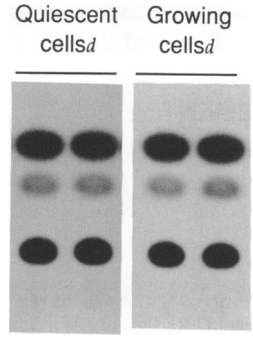
The introduction of foreign DNA sequences into mammalian cells mediated by DNA transfection is a basic method in modern molecular biology. Several different DNA transfection methods are currently used that have varying efficiencies dependent on the type of cell and its growth status. However, none of the available approaches has been shown to lead to transfection of growth arrested cells in culture at an appreciable level. In this report we show that poly-L-ornithine combined with a DMSO shock mediates efficient transfection of both growing and resting cells.

Polybrene (1), a polycation, allows transfection of Chinese hamster ovary (CHO) cells with either plasmid or genomic DNA at an efficiency about 10-fold greater than calcium phosphate mediated gene transfer (2). Because of the relatively high transfection rate observed with polybrene, we examined the relative efficiencies of various other polycations for their ability to transfect CHO cells. In stable transfections using pSV2neo and G418 selection (2) and a similar protocol to that described for polybrene (2), poly-L-ornithine produced the highest rate of transfection ($7.0 \pm 2.2 \times 10^4$ colonies/μg DNA/5×10^5 cells), significantly higher than poly-L-lysine ($1.3 \pm 0.6 \times 10^4$ colonies/μg DNA/5×10^5 cells) which gave comparable results to polybrene ($1.2 \pm 0.6 \times 10^4$ colonies/μg DNA/5×10^5 cells). In transient transfections with pC110 (Pharmacia), a β-galactosidase reporter construct driven by the SV40 promoter, 25.6% of CHO cells were positive for β-galactosidase (3) after transfection mediated by poly-L-ornithine versus 5.0% positive cells with polybrene. In similar transient experiments using a growth hormone gene driven by the mouse metallothionin-1 promoter (pXGH5; Nichols Institute Diagnostics) the amount of growth hormone secreted was 30ng/ml for DEAE–Dextran mediated gene transfer and >300ng/ml for poly-L-ornithine. Thus, poly-L-ornithine is about 5–10 times more efficient for both transient and stable transfections of this cell type than other standard methods of gene transfer. Based on these data, we investigated the usefulness of poly-L-ornithine for transient transfection of quiescent CHO cells in serum depleted media.

Quiescent cultures of CHO cells were prepared using a growth controlled flat revertant CHO cell line, LR-73, which was shown to arrest in G1 with the maintenance of a high viability for several weeks in medium containing 2% serum (4). The plasmids used were either pH1°CAT, a construct in which the chloramphenicol acetyltransferase (CAT) gene was driven by the mouse histone H1° promoter containing H1° 5′ flanking sequences and 5′ untranslated region from −2800 to +252, or pC110. LR-73 cells were seeded at 2×10^5 cells per 100 mm plate in 10 ml αMEM plus 10% fetal bovine serum (FBS). After overnight incubation, the medium was removed, and the plates were washed twice with

Table 1. Transient expression of two reporter gene constructs in quiescent and growing LR-73 cells

Assays[a]	CAT Activity % conversion/μg protein/min	β-gal Positive Cells (%)[d] Experiment 1	Experiment 2
Quiescent cells	0.08 ± 0.01	3.2 ± 1.2	4.5 ± 1.8
Growing cells	1.38 ± 0.02	26.3 ± 4.1	18.2 ± 2.5



[a]No transfection occurred in the absence of DNA, poly-L-ornithine, or DMSO, and the concentration and chain length of poly-L-ornithine described produced optimal transfection rates.
[b]Assayed with 50 μg cell extract for 15 min at 37°C.
[c]Assayed with 10 μg cell extract for 5 min at 37°C.
[d]Each number represents the averge of three plates of transfected cells (mean ± SD).
[e]Autoradiograph of CAT activity assayed by the standard thin layer chromotography in transiently transfected quiescent and growing LR-73 cells. Results from duplicate transfections.

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αMEM containing 1% FBS, and incubated in αMEM plus 1% FBS for at least 5 days before transfection. On the day of transfection, the medium was replaced with 3 ml αMEM plus 1% FBS containing 10 μg plasmid DNA and 10 μg/ml of poly-L-ornithine (Sigma, P3655), dispensed from a 10 mg/ml sterile water stock solution, and incubated at 37°C with gentle mixing every 1.5 hours. After 6 hours, the medium was aspirated and replaced with 30% (v/v) DMSO in αMEM plus 1% FBS for exactly 4 minutes at room temperature. The plates were then rapidly washed and 10 ml fresh αMEM with 1% FBS was added to each plate. For transfecting growing cells, LR-73 cells were seeded at 5×10^5 per 100 mm plate in αMEM plus 10% FBS 16 hours prior to transfection. The transfection was performed exactly as described for stationary cells except all procedures were carried out with medium containing 10% serum. In both cases, after an additional 48 hours of culture, cells were either fixed and stained for β-galactosidase activity or harvested to prepare extracts for CAT assays (5, 6).

Comparison of the transfection efficiencies for both CAT and β-galactosidase constructs in quiescent and growing cells are summarized in Table 1. The results indicated that the transient transfection efficiency with poly-L-ornithine was considerably higher (4−15 fold) for proliferating cells relative to resting cells. This is unlikely to be due to changes in promoter activity since the H1° gene is not induced upon serum stimulation (7). Despite this lower activity, expression of the transfected genes was readily detectable using both plasmid constructs in quiescent cells. In the CAT assay nearly 60% of the chloramphenicol was converted by incubation with 50 μg protein of quiescent cell extracts for 15 min (Table 1). In addition, the number of β-galactosidase staining positive cells was also consistently above a few per cent of cells for the serum starved cells. In contrast, using standard protocols for transient expression it often requires 50−100 μg extract protein for 30−120 min. of incubation to reach a comparable level of CAT activity even for growing cells (5, 6).

In conclusion, poly-L-ornithine when combined with a DMSO shock mediates efficient transfection of CHO cells. Importantly, we report for the first time the ability to directly and effectively transfect cells in a growth arrested state. The method has also proven effective for other cells including rat embryo fibroblasts (REF) in both stable and transient transfections, and should provide a simple and inexpensive way to transfect many adherent cell types and to study regulation of exogenous gene expression during serum stimulation and the cell cycle.

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