Transfection of H4IIE and HEK293 cells with METAFECTENE

Brigitte Parlow and Dieter Schmoll

Department of Medical Biochemistry and Molecular Biology, Ernst-Moritz-Arndt University Greifswald, D-17487 Greifswald, Germany; schmoll@mail.uni-greifswald.de

The transfection of mammalian cells with reporter gene constructs is an often used method for the study of gene function, like the analysis of a gene promoter or of signalling pathways regulating the gene transcription. In this context, it is desirable to achieve a high expression level of the gene products with a minimal amount of DNA. In the present study, we compared the transfection of the mammalian cell lines H4IIE and HEK293 by METAFECTENE with that by the DNA/calcium phosphate co-precipitation method. The rat hepatoma cell line H4IIE (ATCC CRL-1600) has been used by several groups for the analysis of hepatic gene function. In comparison to e.g. HepG2 cells, H4IIE cells have the advantage that they are responsive to physiological insulin concentrations, thus allowing the study of intracellular signalling pathways, which mediate the action of insulin on hepatic gene expression. However, H4IIE cells are difficult to transfect with conventional DNA/calcium phosphate co-precipitation methods and require high amounts of DNA to allow the detection of the expressed gene product. The human adenovirus 5-transformed Human Embryonic Kidney 293 cell line (HEK 293, ATCC CRL-1573) are often used to study biological processes, like signalling events, and also to package and propagate recombinant adenoviral-based vectors.

In order to optimize the transfection of H4IIE und HEK293 cells with METAFECTENE the manufacturer's protocol for the 12-well format was carried out. The cells were transfected with a luciferase reporter gene construct. DNA and METAFECTENE were diluted in DMEM without serum and antibiotics. The cells were cultivated in DMEM/10 % serum and in the presence of penicillin and streptomycin. 18 h after the transfection the DNA/liposome complex was removed and replaced by serum free DMEM. Luciferase reporter gene activity was measured in cell lysates 46 h after the transfection. H4IIE and HEK293 showed the highest reporter gene activities using the combination of 0.5 µg DNA and 2 µl METAFECTENE (Fig. 1) and 0.5 µg DNA and 1 µl METAFECTENE, respectively (Fig. 2).

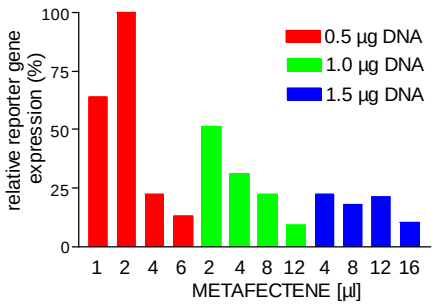

Fig. 1 Transfection of H4IIE cells

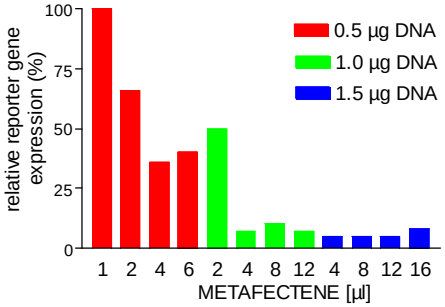

Fig. 2 Transfection of HEK293 cells

Next, H4IIE cells and HEK293 cells were transfected in parallel following the respective optimized METAFECTENE protocol or the DNA/calcium co-precipitation protocol (1), routinely carried out in our laboratory, which uses 3.5 µg DNA and 1.2 µg DNA per 12-well dish for H4IIE cells and for HEK293, respectively. The cells were incubated for 18 h with the DNA/calcium phosphate complex. H4IIE cells were subsequently glycerol-shocked (15% glycerol, 2 min) to increase the transfection efficiency. Reporter gene assays were performed 46 h after the transfection. As shown in Fig. 3 and 4, reporter gene activities were approximately 5 times higher in METAFECTENE-transfected cells than in DNA/calcium phosphate transfected cells, although less DNA was used.

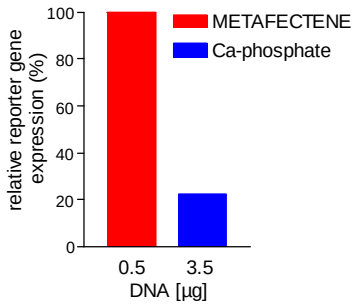

Fig. 3 Transfection of H4IIE cells

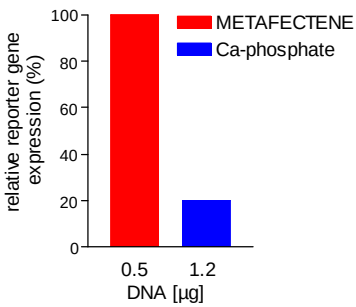

Fig. 4 Transfection of HEK293 cells

In conclusion, METAFECTENE allows the efficient and rapid transfection of H4IIE and HEK293 cells without the need of additional reagents or experimental steps, like the incubation with glycerol. METAFECTENE should be especially advantageous if high transfection efficiencies and expression levels are required, if the DNA amounts are limited (e.g. low copy plasmids) or if the expression of the gene product is driven by a weak promoter.

(1) Docherty, K. and Clark, A.R. (1993) in: Gene Transcription: A Practical Approach (Hames, B.D. and Higgins, S.J., Eds.), pp. 71-73, IRL Press, Oxford.