Gene delivery by METAFECTENE EASY to the intestinal fragments for organ culture

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Introduction:

We have been studying the mechanisms of intestinal remodelling that occurs during *Xenopus laevis* (African clawed frog) metamorphosis, which is triggered by thyroid hormone (TH) (Shi, 1999). TH binds to its cognate receptor in the nucleus, then the ligand-receptor complex activates expression of the target genes by binding to their promoter region. Thus, an important approach to study the molecular basis of intestinal remodelling has been to analyze the TH response genes. To investigate functions of the genes of interest, it is necessary to utilize the gene delivery system such as transgenesis for in vivo and transfection for in vitro studies. In our laboratory, the gene delivery system by electroporation has been established for in vitro organ culture experiments (Ikuzawa et al., 2006). However, electroporation is time-consuming, laborious and sometimes damages the tissues. Here I show that the plasmid DNA driving EGFP was successfully delivered into the intestinal fragments by METAFECTENE EASY, indicating its usefulness for the future studies.

Materials and methods:

The anterior part of the small intestine was isolated from prometamorphic tadpoles. The intestine was open lengthwise and cut into 4-5 mm long. They were washed with the culture medium that consists of 60% Leibovitz's L-15 medium containing 10% FBS, 5 μg/mL Insulin, 0.5 μg/mL Hydrocortisone, 100 IU/mL penicillin and 100 μg/mL streptomycin (Ishizuya-Oka and Shimozawa, 1994). METAFECTENE EASY was kindly provided by Biontex Laboratories. Plasmid DNA used for transfection was pIRES2_EGFP (Ikuzawa et al., 2006).

Experimental procedures / transfection protocol:

15-20 intestinal fragments were immersed in 500 μL of the culture medium in a 1.5 mL tube. Lipoplex containing pIRES2_EGFP was prepared with 5 μg of DNA according to the manufacture's instructions. For a control experiment, the naked plasmid DNA was used. 100 μL of the solution containing Lipoplexes or naked plasmid were added to the 1.5 mL tube containing the intestinal fragments, thoroughly and gently mixed, transferred into a 24-well plate and incubated overnight at 26°C to perform transfection. Then, the intestinal fragments were placed on a Transwell (Corning) set in a 6-well plate and cultured 2 more days at 26°C. The intestinal fragments were washed with PBS followed by RNA extraction. cDNAs for rpL8 (loading control) and EGFP were amplified by using One-step RT-PCR kit (Invitrogen).

Results and discussion:

Expression of EGFP was check by RT-PCR. Although EGFP was faintly expressed in the intact samples that were cultured with the naked plasmid DNA (Fig. 1, lane 2), EGFP was markedly expressed in the transfected intestine (Fig. 1, lane 3). These results indicated that the plasmid DNA in the Lipoplex was successfully delivered.

Conclusion / summary:

I showed that plasmid DNA was successfully delivered by the action of METAFECTENE EASY. Although the morphology of intestinal fragments was not retained normal (not shown), this is due to the culture condition because all samples showed same morphology. It is necessary to overcome this problem, but the results so far imply that METAFECTENE EASY can be a powerful tool for studying gene functions in the organ culture system.

References:

Ikuzawa M, Shimizu K, Yasumasu S, Iuchi I, Shi Y-B, Ishizuya-Oka A. 2006. Thyroid hormone-induced expression of a bZip-containing transcription factor activates epithelial cell proliferation during *Xenopus* larval-to-adult intestinal remodeling. Dev Genes Evol 216:109-118.

Ishizuya-Oka A, Shimozawa A. 1994. Inductive action of epithelium on differentiation of intestinal connective tissue of *Xenopus laevis* tadpoles during metamorphosis in vitro. Cell Tissue Res 277:427-436.

Shi Y-B. 1999. Amphibian Metamorphosis: From morphology to molecular biology. New York: John Wiley & Sons, Inc.

Appendix: Tables and/or figures:

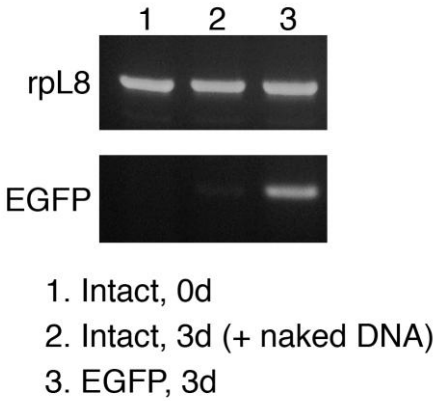

Fig. 1. Gene delivery into the intestinal fragments using METAFECTENE EASY. Total RNA was extracted from untreated intestinal fragments before cultivation (lane 1. Intact, 0d), intestinal fragments cultured with naked DNA for 3 days (lane 2. Intact, 3d + naked DNA) and EGFP-transfected intestinal fragments (lane 3. EGFP, 3d). RT-PCR was performed to amplify cDNAs for rpL8 (loading control) and EGFP. Marked expression of EGFP was detected only in the transfected intestinal fragments.