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Improvement of Transfection Efficiency of Epithelioma Papulosum Cyprini Carp Cells by Modification of Cell Cycle and Use of an Optimal Promoter

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Abstract: Several methods to improve transfection of epithelioma papulosum cyprini (EPC) carp cells have been tested and are reported here. By modifying the cell cycle state of EPC cell monolayers and selecting the best promoter for the plasmid to be transfected, we increased transfection efficiency from 12.8% to 55.1% and decreased the coefficient of variation among different experiments from 54.1% to 11.8%. Thus 2- to 3-fold higher transfection efficiencies were obtained when the EPC monolayers were treated with colchicine or thymidine before transfection. In addition, the plasmids pMOKβgal and its shorter derivative pMVC1.4βgal, both containing 218 bp of additional sequences upstream of the cytomegalovirus promoter contained in plasmid pCMVβ, consistently produced higher transfection efficiencies than pCMVβ. Combination of the two methods resulted in an improvement of both efficiency and reproducibility. These results should facilitate transfection of EPC cells to use as a model to obtain transgenics, to conduct quantitative transfected-cell fusion assays, to improve DNA-immersion-vaccination methods, or to obtain infectious cDNA from fish RNA viruses.

Key words: epithelioma papulosum cyprini (EPC) cells, carp, transfection.

INTRODUCTION

Because fish cells have longer cell cycles than mammalian cells and lower optimal temperatures for growth, commercial transfection reagents based on liposomes and developed for mammalian cells are not optimal for fish cells. However, attempts have been reported to optimize plasmid introns (Betancourt et al., 1993), promoters (Inoue et al., 1990; Moav et al., 1992; Sharps et al., 1992), enhancers (Friedenreich and Schartl, 1990), oncogenes (Hayasaka et al., 1990), or use of multipotent fish cells (Bejar et al., 1999) for transfection of fish cell lines (Hackett and Alvarez, 2000; Bearzotti et al., 1992).

Because the cell line epithelioma papulosum cyprini (EPC), isolated from carp (Fijan et al., 1983), was found to be the best predictor of plasmid activity in transgenic fish (Moav et al., 1992) and could be transfected (Bearzotti et al., 1992; Moav et al., 1992), we have used it as a first model to study possible methods to improve the previously

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