EFFICIENCY OF GENE TRANSFER AND PROMOTER SPECIFICITY ASSAYED BY TRANSIENT GENE EXPRESSION IN ZEBRAFISH

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Introduction

The increasing importance of aquaculture in future food production potentiates the need for developing an increased knowledge in the molecular biology of aquatic organisms. Fish transgenesis represents both a tool for basic research and a possibility for improving stocks of production animals. The zebrafish *(Danio rerio)* is a widely used laboratory model for such research.

Results

The *E. coli* β-galactosidase gene (LacZ) and the firefly luciferase gene (LUC) were used as reporters of gene expression in zebrafish.

In order to dissicate the salmon GnRH promoter region (1) into functional sequence elements, subsets of sGnRH LacZ fusion genes were made. Results from microinjection of the salmon GnRHLacZ fusion gene showed tissue specific LacZ expression by x-gal staining at the 48 hr stage of zebrafish development. At earlier stages (12 and 24 hr) the GnRH promoter directed expression but showed less tissue specificity. A cytomegalovirus (CMV) promoter LacZ fusion gene control directed reporter expression in many cell types of the zebrafish embryo.

SV4O large T antigen nuclear localization sequence (NLS) (2) pCMV-LUC DNA complexes were analysed by gel retardation experiments resulting in band shifts at a molar ratio of 100:1. Various concentrationss and molar ratios of NLS-pCMVL complexes were microinjected into the cytoplasm of zebrafish eggs and nuclear uptake was monitored as LUC expression. NLS was shown to facilitate DNA targetting to the nucleus by > 100 times. When NLS was replaced by a reverse NLS peptide, it was shown that the peptide-DNA binding was of similar strength but that facilitated transfer of pCMVL to the nucleus seen with NLS was abolished with reversed NLS (Collas *et al.*, manuscript submitted).

Discussion

The salmon GnRH promoter showed a high degree of tissue specificity in transient expression when microinjected in zebrafish eggs. Facilitated transfer of DNA from the cytoplasm to the nucleus when complexing the injected DNA with NLS, should theoretically potentiate integration of foreign DNA earlier than with naked DNA. If so, a reduced degree of mosaicism might occur. This assumption is at present under investigation in our laboratory

Experimental procedures

Zebrafish were maintained as described (Westerfield 1993) (3). Microinjection of 250 pl of DNA solution was carried out into dechorionated zebrafish eggs at 1-2 cell stage. Luciferase expression was monitored at 4, 24 and 48 hr after injection of pCMVL (4) and β-galactosidase activity from pPab-LacZ (Husebye upublished) was determined with x-gal staining at 12, 24 and 48 hr pi. Binding of NLS (CGGPKKKRKVG-NH_2)and reverse NLS (GGGGVKRKKKP-NH_2) to plasmid DNA was achieved in 0.25 M KCl at room temperature.

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