ECTOPIC EXPRESSION OF tiGH IN MUSCLE CELLS OF TRANSGENIC F2 TILAPIAS

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Introduction

Over the past decade, the technique of transferring foreign DNA into fertilized eggs by microinjection or electroporation has become the most popular method for generating a wide range of transgenic fish species. The transgenic technology offers an excellent opportunity for conducting research in basic science as well as biotechnological applications. In fish, the most common biotechnological approach has been the growth enhancement as a strategy to shorten long production cycles. In our laboratory we have develop a transgenic tilapia line, that carry an additional copy of tilapia growth hormone (tiGH) under the regulation of a CMV promoter (1). In the F2 progeny study (*albino* line) (2), we have demonstrated the transgene RNA and protein expression in muscle and gonads. In this paper we characterized the ectopic tiGH expression in muscle cells.

Materials and Methods

DNA samples were extracted from the F2 progeny (transgenic female X transgenic male) and were assayed for the presence of transgene sequences by PCR amplification (2).

The study of RNA expression was carried by in situ hybridization using an oligonucleotide probe encoding a fragment of tiGH cDNA (antisense-probe 5' CTACAGAGTGCAGTTTGCT-TCTGGAGA 3' and sense-probe 5' TGTCTGGAGGTTTCCTCTCTGAGGAAC 3'). The muscle of transgenic tilapias was fixed in 4 % paraformaldehyde in PBS at room temperature for 30 minutes, washed twice in PBS for 5 minutes each and after freezing the tissue, serial transversal sections were cut at 10 mm thickness. Several adjacent sections were mounted on a gelatine-coated slide for the *in situ* hybridization and immunocytochemistry staining. The tissue section for *in situ* hybridization was post-fixed 5 minutes, washed twice in PBS for 5 minutes each, in 2X SSC for 10 minutes and preincubated for 2 hours with a hybridization buffer (50 % formamide, 10 % dextrane sulfate, 5X Denhardts, 2X SSC and 25 mg/ml of yeast tRNA). The probe was labeled with DNA Tailing Kit (Boerihnger, Germany), using digoxigenin (DID-11-dUTP). The probe was applied diluted (1:200) in hybridization buffer and incubated overnight at 37 °C in a moist chamber. They were then washed sequentially with 2X SSC for 30 min, with 1 x SSC for 30 min, with 0,5X SSC for 30 min at room temperature and with 0,5X SSC for 30 min at 37 °C. The sections were then processed for immunological detection with Anti-digoxigenin-AP, Fab fragment (Boerihnger, Germany). Immunostaining was carried out according to Inostroza *et al.*, 1990 (3) using as first antibody a rabbit anti-serum to rtiGH. Rabbit antibodies to carp vitalogenin were used as control. The second antibody was a goat. Anti-rabbit IgG-FITC conjugate (SIGMA, USA).

Results and Discussion

The fish growth hormone is a secreted hipophisial hormone that is involved in the control of growth (4). Figure 1 shows the expression of the CMV-tiGH transgene in muscle cells from transgenic F2 tilapia. Chimeric construction was able to produce RNA and protein in muscle tissues of transgenic tilapias. This Ectopic tiGH expression was probably the responsible for the growth acceleration in these tilapias.

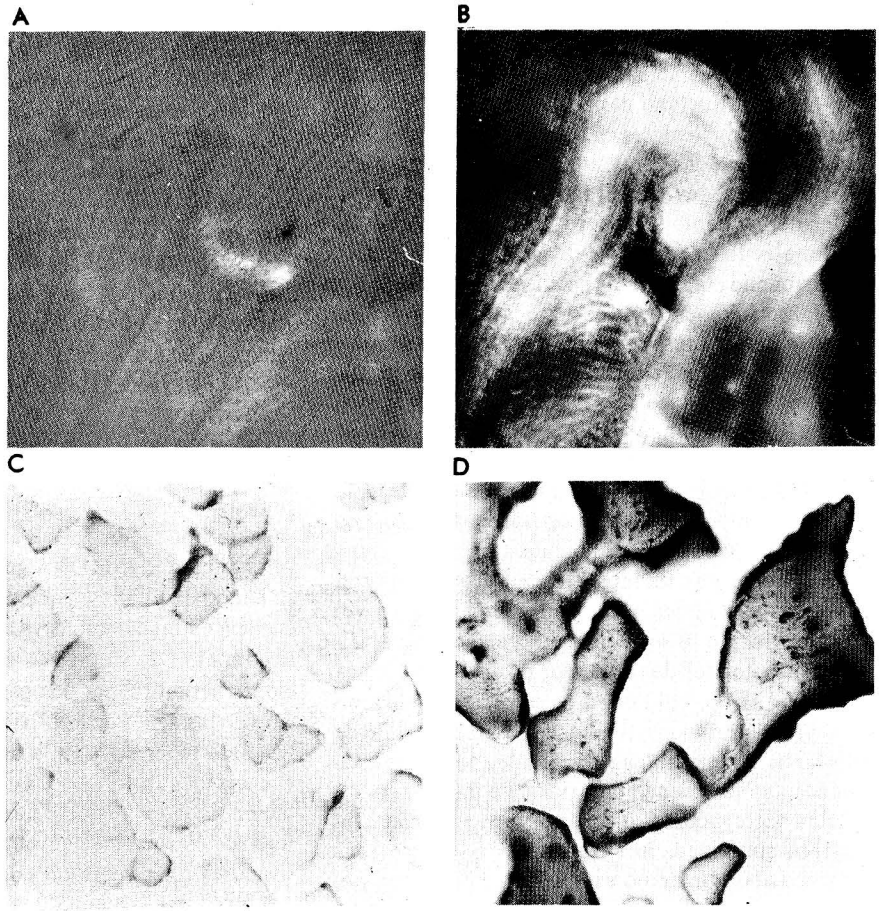

Figure 1. In situ *hybridization and immunocytochemistry of transversal section of transgenic tilapia muscle. A and B.* In situ *hybridization with digoxigenin-labeled oligonucleotide sense (A) and antisense (B) probe for tiGH. C and D. Immunocytochemistry of transversal section of transgenic tilapia muscle developed with an anti-carp vitalogenin sera (C) and with an anti-tilapia growth hormone sera (D).*

Acknowledgments

This work was partially supported by international Center for Genetic Engineering and Biotechnology Collaborative Research Programme (project CRP/CUB93-05).

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