GENE TRANSFER FOR GROWTH MANIPULATION IN TILAPIA (Oreochromis spp)

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Tilapia are economically important species accounting for over 67 % of the fresh water fish production in Cuba. The possibility of accelerating growth in tilapia was assayed by exogenous administration of recombinant tiGH. The growth of juvenile tilapia (*Oreochromis* spp.) was accelerated (1,4 fold in length and 1,7 fold in body weight after 21 days) when a *Pichia pastoris*-derived tiGH was administrated by three intraperitoneal injections at intervals of 7 days. The control group received BSA injections.

For gene transfer experiments, chimeric constructs were prepared containing the tilapia growth hormone cDNA (tiGH) or chromosomal gene (chr-tiGH), 5' regulatory sequences derived from the human cytomegalovirus (CMV) or Rous sarcoma virus (RSV), polyadenylation sites from the SV40 and the first intron from the trout growth hormone gene (INT) (1-3). Employing these constructs, tiGH transient expression was obtained in mammalian and/or fish cells (1-3). Transient expression experiments with these regulatory sequences supported the hypothesis that different regulatory requirements exist in cells and embryos and suggested that chimeric constructs designed for transgenic experiments should be assayed in transient expression experiments in fish embryos (4). To evaluate the activity of different regulatory elements in transgenic fish, five lines of transgenic tilapia were generated (I: CMV >tiGH>CAT>SV40, II: CMV>+INT>tiGH>SV40, III: CMV>-INT>tiGH>SV40, IV: RSV+INT>tiGH>SV40, and V: RSV>chr-tiGH) by direct microinjection of one cell embryos (5). With construct I, a transgenic animal containing 1 copy of the transgene per cell was selected to establish a transgenic line (F0-3 in (1) and afterwards designated as *albino*) (5). The transgene was stably transmitted to F1, F2 and F3 generations in a Mendelian fashion. Ectopic, low level expression of tiGH was detected in gonad and muscle cells of F1 transgenic tilapia by *in situ* hybridization and immunohystochemical analysis of tissue sections. Nine month old transgenic F1 progeny were 82 % larger than non-transgenic at p = 0,001. These results showed that low level ectopic expression of tiGH resulted in a growth acceleration in this transgenic tilapia line. With the II-V constructs, transgenic tilapia were generated carrying 8->50 copies of the transgene/genome. Transgenes were transmitted to F1 progeny in a Mendelian fashion.

Transgenic F1 tilapia with transgenes II-IV, showed only a moderate increase in weight [II: 3,4 % (p = 0,0057), III: 3,3 % (p = 0,8) and IV: 5,0 % (p = 0,1)] when compared to non-transgenic siblings. We are now characterizing the ectopic tiGH expression levels in these transgenic tilapia to establish a correlation with their growing phenotype. Although hormones generally work at low concentrations, we need to compare the growing phenotype of transgenic lines obtained with different tiGH-containing chimeric constructs. Since we do not know the optimum constitutive GH levels required for better growth increase in tilapia, results have to be obtained empirically comparing different constructs and transgenic lines in heterozygous and homozygous animals, characterizing the tissues and levels of ectopic tiGH expression.

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1. Martínez *et al.* Miami Short Reports 1992;2:53.
2. Hernández *et al.* Biotecnología Aplicada 1993a;10:158-161.
3. Hernández *et al.* Molecular Marine Biology and Biotechnology 1993b;2(3):181-188.
4. García del Barco *et al.* J. of Marine Biotechnology 1994;1:203-205.
5. De la Fuente *et al.* J. of Marine Biotechnology 1995;(in press).