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Using Quantitative PCR to Study Insulin-like Growth Factor in Goldfish

All vertebrates control the rate at which their bodies grow by employing a family of peptides called insulin-like growth factors (IGFs). The way in which IGFs are mobilized has been conserved as the vertebrates have evolved. In fishes IGF-I production is stimulated by the pituitary growth hormone GH, and in mammals a similar pattern of IGF-I stimulation by GH is seen. The amino acid and nucleotide sequence of IGF-I has been determined in a number of vertebrate species, including frogs, chickens, a dozen kinds of mammals, and seven kinds of fish. The IGF-I sequence is highly conserved among this diverse group of vertebrates.

The great similarity of IGF-I growth factors among such a broad array of vertebrates, and their common mode of stimulation by GH hormones, raises an intriguing question. Can the pituitary hormone from one species function to activate the IGF-I transcription factor in another?

This question has been investigated as part of a larger research program by Hamid Habibi and Maurice Moloney of the University of Calgary designed to characterize the IGF-I growth control system of goldfish.

The research team first examined goldfish IGF-I in detail. To get a clear picture of the goldfish IGF-I molecule, they cloned and characterized goldfish IGF-I cDNA (a DNA "copy" of the IFG-I mRNA.) The 833 nucleotide sequence of the IGF-I message encodes a 161 amino acid protein that has 97% and 93% similarity to carp and salmon IGF-I respectively, and a high structural similarity with IGF-I molecules in other vertebrates as well. The research team went on to identify three different forms of IGF-I in goldfish liver, and two more in goldfish ovary, each with a slightly different nucleotide sequence.

To assess the ability of GH from other species to stimulate the production of goldfish IGF-I, the research team needed a sensitive way to monitor IGF-I mRNA production. To accurately quantify the relative amounts of IGF-I produced in response to treatment with GH, they developed a sensitive quantitative assay using the polymerase chain reaction (PCR), a method they call competitive quantitative PCR, or Q-PCR.

Hormones in goldfish. Goldfish release the hormone, IGF which is similar to IGF found in other fish.

The general scheme for Q-PCR is that two sequences, sharing the same 3' and 5' ends, compete for the same primer used for amplification in PCR. The more of one sequence is present, the better it will compete for primer, resulting in far greater amplification. In this way, small quantitative differences in two sequences can be hugely amplified.

In the procedure, two nucleotide strands, the control "template" sequence and the cell-derived mRNA for IGF-I, are amplified through 25 cycles of PCR, competing at each cycle for the same primer. The DNA that results is then separated by electrophoresis into two bands, and their densities measured. The more mRNA from the goldfish IGF-I gene, the greater its band density and the lower the ratio of template sequence band density to IGF-I cDNA band density. By plotting the ratio of the band densities (template/IGF-I) against the concentration of template added at each PCR cycle, the researchers could determine the relative amounts of IGF-I mRNA produced when goldfish were stimulated with GH from other fish species.