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Molecular cloning of HSP90 gene from flounder *(Paralichthys olivaceus)*

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Introduction

Once cells are exposed to heat and a variety of other stressful stimuli, the highly conserved heat shock proteins (HSP) are accumulated. Heat shock proteins which function mainly as molecular chaperones, allow cells to adapt to gradual changes in their environment and to survive in otherwise lethal conditions. HSP90 has been reported to function in signal transduction and the maturation and the enhancement of affinity against glucocorticoid receptors. These molecular chaperones play a major role in the conformational maturity and stabilit of several signaling molecules, including steroid recrators and, therefore, are thought to be important mediators of intracellular signaling events (Csermely et al,. 1998).

Materials and Methods

Isolation of total RNA and poly A+ RNA

Total RNA was isolated using TRIzol reagent (Gibco).

Construction of flounder pituitary cDNA library

A pituitary cDNA library was constructed from pituitaries that were obtained from male and female flounder at all reproductive stages. cDNA library was constructed as described in the Manufacture's instruction (Stratagene, cDNA library construction kit).

Synthesis of flounder hsp90 and actin cDNA using PCR

A 0.3 kilobase (kb) fragment of hsp90 was amplified by polymerase chain

reaction (PCR) for use in screening a cDNA library. The target cDNA was amplified by PCR using the following degenerate primers incorporated at the 5′ end: 5′-GACACTTCTTGACGATGTTCTTG-3′and LT3 primers. PCR products were analyzed by electrophoresis in a gel of 1.0% agarose in TBE buffer and 0.1% ehidium bromide.

Purification of plasmid DNA and Sequence analysis

The clone obtained from screening of library was purified using Wizard Plus SV minipreps DNA purification system (Promega) and checked for the size of the inserted cDNA. The nucleotide sequences of cloned cDNAs were determined by a chain-termination method using BigDye terminator premix kit (Perkin Elmer). Sequencing was carried out using SP6 and T7 Universal primers. The fluorescence-labeled nucleotides were analyzed on an ABI PRISMTM 310 automatic sequencer (Perkin Elmer).

Results and Conclusion

We obtained a partial nucleotide sequence for flounder HSP90 by PCR using highly conserved regions of chinook salmo and zebrafish HSP90 cDNA. The sequence consisted of 284 bases (Fig. 1) and showed high sequence homology with chinook salmon (98%), zebrafish (84%), and human (77%) HSP90 cDNAs.

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631     GAGGGTTAGG GAGATTGTGA AGAAGCACTC TCAGTTTATC GGCTACCCCA
681     TCACCCTGTT TGTGGAGAAG GAGCGTGACA AGGAGATCAG TGACGACGAG
731     GCAGAGGAGG AAAAGGCTGA GAAGGAGGAG AAAGAGGATG AAGGTGAGGA
751     CAAGCCAAAG ATTGAGGATG TTGGGCTCAG ATGATGAGGA AGACTCCAAA
801     GACAAGGACA AGAAGAAGAC AAGANGATCA AGGAGAAGTA CATCGTCCAG
851     GAGGAGCTGA ACATNACCAA GCCCCATCTG GACC
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Fig. 1. Open reading frame of the partial nucleotide sequence of HSP90 cloned from flounder.

References

Csermely, P., Schnaider, T., Soti, G., Prohaszka, Z., Nardai, G., 1998. The 90-kDa molecular chaperone family structure, function, and clinical applications. A comprehensive review. Pharmacol. Hter, 79, 129-168.