

Molecular cloning of Carboxypeptidase H genes from flounder

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Introduction

Carboxypeptidase H (CPH) is a putative post-translational processing enzyme, which removes basic amino acid residues from intermediates during protein-hormone biosynthesis. CPH is a metallo-carboxypeptidase implicated in the processing of peptide hormones (Loh, *et al.* 1984; Fricker *et al.* 1986). In the rat brain, CPH is transcribed from a single transcriptional start site associated with the initiator-type element first described in the gene encoding lymphocyte-specific terminal deoxynucleotidyltransferase.

Materials and Methods

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 Manufacturer's instruction (Stratagene, cDNA library construction kit).

Screening of the flounder pituitary library

The PCR probe was labeled with Dig(Digoxigenin)-11-dUTP. The rest hybridization method followed the procedure of ZAP-cDNA Synthesis Kit (Stratagene) manual.

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 T3 and Universal primers. The fluorescence-labeled nucleotides were analyzed on an ABI PRISM™ 310 automatic sequencer (Perkin Elmer).

Results and Conclusion

The gene encoding flounder CPH was cloned from a flounder pituitary cDNA library (Fig 1). The nucleotide and peptide sequences were compared to those of CPH genes from other species. It showed a sequence homology as high as 87% in anglerfish. We also analyzed a phylogenetic tree based on CPH amino acid sequences from various species.

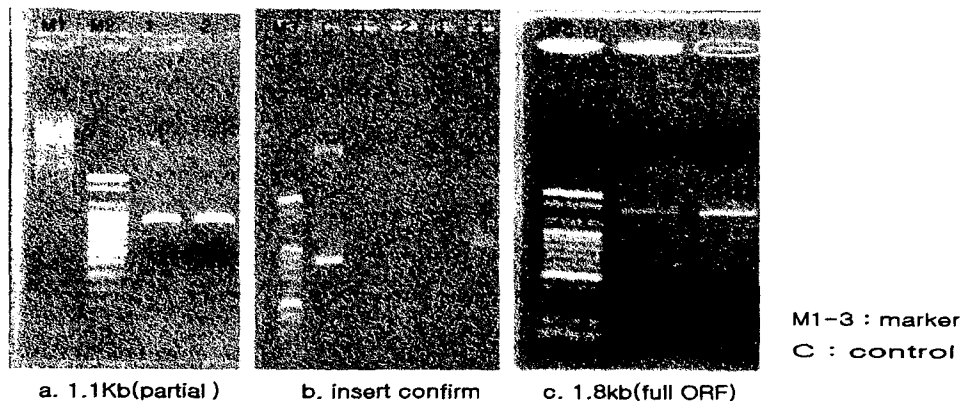


Fig 1. PCR of CPH gene from flounder pituitary cDNA library.

References

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