Asian Fisheries Science **14** (2001): 353-356
Asian Fisheries Society, Manila, Philippines

cDNA Cloning of Polypeptide Chain Elongation Factor 1α from Yellowtail *Seriola quinqueradiata*

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Abstract

Polypeptide chain elongation factor 1α (EF1α) was cloned from the kidney tissues of yellowtail *S. quinqueradiata*. The entire open reading frame of EF1α was 1386 bp long. Deduced amino acid sequences of yellowtail EF1α showed a high similarity (more than 87% identity) with those of other vertebrates. Yellowtail EF1α had one amino acid deletion at the position of 447 amino acid of other vertebrate EF1α and the ribosome-binding and GTP binding domains were well conserved.

Introduction

Yellowtail is the most important fish species in Japanese marine culture, and the products reach 150,000 tons per year. However, the molecular biological studies on this species are few and the DNA sequences registered in DNA database are less than 10.

Elongation factor 1α (EF1α) is one of the cytoplasmic proteins and an essential component of the protein synthetic complex that promotes the binding of aminoacyl-tRNA to ribosome in a GTP-dependent manner (Moldave et al. 1985). Additional functions of EF1α are, its interaction with actin (Yang et al. 1990), mitotic apparatus (Ohta et al. 1990) and the endoplasmic reticulum (Hayashi et al. 1993). In fish species, EF1α gene was investigated only in zebrafish *Danio rerio* (Gao et al. 1997) and medaka *Oryzias latipes* (Kinoshita et al. 1999), partial DNA sequences registered in Genbank (rainbow trout, U78974; brown trout, U29205). In this study, cDNA of EF1α from yellowtail was cloned and characterized for future investigation of the gene structure of EF1α in yellowtail.

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Materials and Methods

Yellowtail weighing about 150 g, was obtained from Ehime Prefecture Fisheries Station, Japan. Total RNA was isolated from 0.3 g of yellowtail head-kidney by ISOGEN (Nippongene). Poly(A)+RNA was isolated using the Quick Prep Micro mRNA Purification Kit (Amersham Pharmacia Biotech) and used for cDNA library construction.

A cDNA Library was constructed using Lambda ZAP II CLONING KIT (Stratagene) and Gigapack II Gold Packaging Extract (Stratagene) according to the manufacturer's instruction.

Yellowtail EF1α partial genes were detected through the Expressed Sequenced Tag (EST) analysis of yellowtail head-kidney cDNA library.However, the sequences determined by EST analysis were missing 5' and 3' prime sequences. Thus, the complete sequences were determined using the 5'-full RACE core Set (TAKARA) following PCR primers:

5' terminal phosphorylated RT primer:
5' - ATTACCCTCCTTGCG - 3';
Sense: 5'-TGGATGGCACGGAGACAACAT - 3',
5' - AAAGATGGGCTGGTTCAAGGG - 3';
Anti-Sense: 5' - ATGGGGACAAAGGCAACAGTG - 3',
5' - GGGTTGTAGCCGATCTTCTTG - 3',
5' - TCAAACTCACCAACACCAGCG - 3',
5' - ACGATCAGCACAGCGCAGTC - 3'

The DNA sequencing reaction was performed using the Big-Dye Terminator cycle sequencing FS ready reaction kit (PE Applied Biosystems) while DNA sequences were determined using ABI prism 310 Genetic Analyzer (PE Applied Biosystems).

Phylogenetic trees were constructed using the Maximum Parsimony Method in the PAUP*4.0 computer program (Sinauer Associates, INC. Smithsonian Institution 1998).

Results and Discussion

The 1386 bp DNA from position 1 to 1386 was identified as an EF1α-encoding open reading frame (Fig. 1). At the DNA sequence level the coding region is 91% identical to medaka, 86% to zebrafish and 78% to human cDNAs.

Multiple alignment of deduced amino acid sequences indicates 96%, 89%, and 87% identities between yellowtail and medaka, zebrafish and human, respectively. The domain interaction with the ribosome (from position 51 to 72) is identical in all vertebrates. The three GTP binding domains (from position 12 to 21, from 88 to 100 and from 148 to 156) were also identical in all compared animals and only the fourth domains showed two amino acid differences between fish and human (from 286 to 300) (Fig.2).

These results indicate the importance of these domains on the function of EF1α. The actin binding domain (from position 161 to 180) is also well conserved in yellowtail EF1α (Fig. 2), compared to that of medaka and zebrafish. Kinoshita et al. (1999) reported that in the latter half of medaka EF1α, 15 of 17 unique amino acid changes were observed, compared with zebrafish and other vertebrates. The sequences in this part of yellowtail EF1α were almost identical to that of medaka. Yellowtail EF1α has one amino acid deletion at position 447. This deletion was also seen in medaka EF1α (Kinoshita et al. 1999).

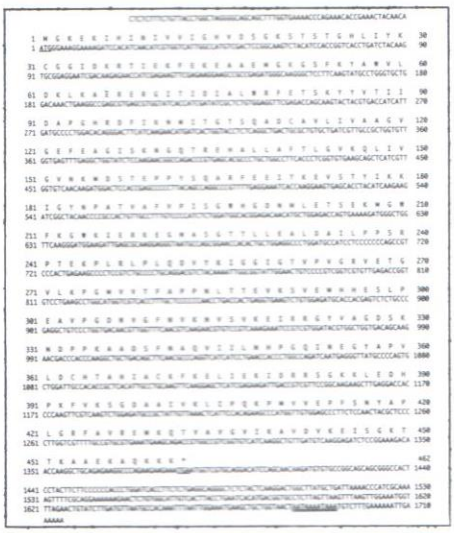

Fig. 1. The nucleotide sequence of the cDNA encoding yellowtail EF1α and deduced amino acid sequence. The start codon (ATG), stop codon (TGA), and poly-adenylation signal sequences (AATAAAATAAA) are underlined. The nucleotide sequence has been assigned DDBJ, EMBL, and GenBank accession No.AB032900.

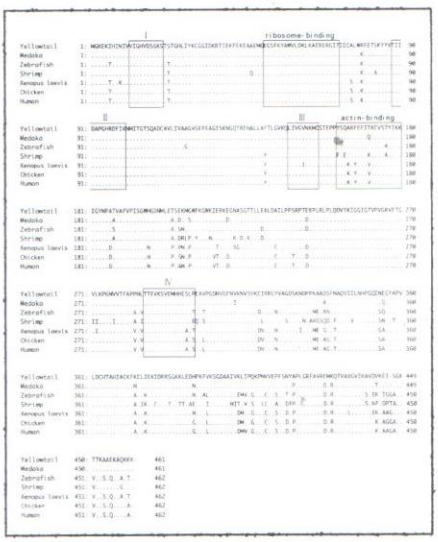

Fig. 2. Comparison of amino acid sequence of yellowtail EF1α with those of other vertebrates; medaka (AB013606), zebrafish (L23807), *Xenopus laevis* (M25504), chicken (L00677), shrimp (X03349) and human (J04617). Amino acids are depicted using the standard one-letter code. Residues identical to yellowtail are indicated by dashes and gaps are introduced to optimize alignment by dots. The functional regions are indicated by (+) and (-), respectively. The symbols I, II, III, and IV represent GTP-binding domains, respectively. This figure based on Kinoshita et al. (1999) was constructed.

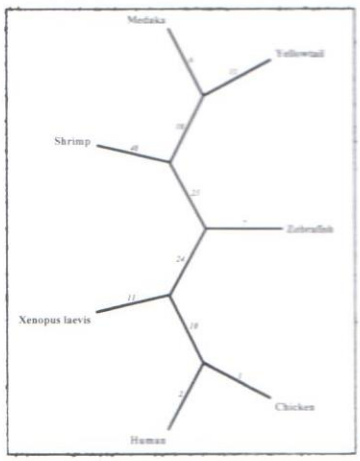

Fig. 3. Phylogenetic tree was constructed from the deduced amino acid sequences of EF1α aligned in figure 2. This tree was obtained following the Maximum Parsimony Method using PAUP * 4.0 computer program (Sinauer Associates, INC. Smithsonian Institution. 1998). The lengths of branches are proportional to the number of molecular changes that occurred.

The cluster analysis is shown in figure 3. The closest animal was medaka, and made up the same cluster. This cluster analysis also reflected the phylogenetic studies based on morphology.

In the studies of transgenic fish, EF1α enhancer-promoter is used for intense transgenic expression in zebrafish as the ubiquitous regulatory element (Lin et al. 1995, Amsterdam et al. 1995). Thus, the analysis of yellowtail EF1α promoter will be useful in making transgenic yellowtail.

Acknowledgments

This work was partly supported by the grant from the Research for the Future Program of the Japan Society for the Promotion of Science (97L00902).

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Manuscript received 08 June 2000; Accepted 28 January 2001