cDNA Cloning of Polypeptide Chain Elongation Factor 1 α from Medaka *Oryzias latipes*

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Polypeptide chain elongation factor 1 α (EF1 α) was cloned from a liver cDNA library of medaka *Oryzias latipes*. The analysis of the clone containing 1.6 kbp DNA revealed that the entire open reading frame of EF1 α was 1383 bp long. Deduced amino acid sequence of medaka EF1 α showed high similarity (more than 87% identity) with those of other vertebrates. Medaka EF1 α had one amino acid deletion at the position of 447 amino acid of other EF1 α s and unique 17 replacement against those of other vertebrates. Northern hybridization analysis revealed that this EF1 α was expressed in all tissues tested.

Key words: polypeptide elongation factor 1 α , EF1 α , cDNA, medaka

Elongation factor 1 α (EF1 α) is one of the most abundant cytoplasmic proteins and an essential component of the protein synthetic complex which promotes the binding of aminoacyl-tRNA to ribosome in a GTP-dependent manner.¹⁾ Additional functions of EF1 α are suggested because of its interaction with actin,²⁾ mitotic apparatus,³⁾ and the endoplasmic reticulum.⁴⁾ Several EF1 α genes are expressed and pseudogenes are also present in many animals and plants.^{5–14)} It is also reported that the expression of different EF1 α genes are developmental stage or organ specific.^{7–13)} For example, in *Xenopus laevis*, there are three EF1 α family genes. One of them (42Sp50) is expressed in immature oocytes, another one (EF1 α O) is expressed in mature oocytes and at early embryogenesis, and the other one (EF1 α S) is expressed during embryogenesis and in somatic cells.⁸⁾ The expression of S1 protein which is a member of the EF1 α family of rat is expressed in brain, heart, and skeletal muscle.¹²⁾ Therefore, it becomes a general concept that the EF1 α family consist of several isoforms whose expression is in a developmental stage dependent manner^{7–11)} and organ specific manner in adult.^{12,13)}

On the contrary, as for fish species, EF1 α gene was investigated only in zebrafish by Goa *et al.*, reporting that only one gene copy of EF1 α per haploid and no pseudogenes was present.¹⁴⁾ The single gene structure of EF1 α per haploid was also reported in chicken.¹⁵⁾ Because EF1 α is one of the most conserved molecule among eukaryotes,²⁾ it

is not easy to accept that this single gene structure of EF1 α observed in zebrafish is common in fish. To clarify this point, the structure of EF1 α gene of other fishes should be investigated.

Medaka, as well as zebrafish, is advantageous as an experimental model in respect to, for example, its short life cycle and established inbred strains.¹⁶⁾ In this study, cDNA of EF1 α was cloned and characterized from medaka *Oryzias latipes* for the future experiment to investigate the gene structure of EF1 α in medaka.

Materials and Methods

Materials

An inbred medaka strain (HNI-I) which was genetically identical¹⁶⁾ was kindly supplied from Dr. K. Ozato and Dr. Y. Wakamatsu (Nagoya university).

Probe for cDNA Library Screening

A degenerate oligo primer pair was designated from well-conserved amino acid sequence of GTP/GDP binding domain of EF-1 α (sense: 5'-CGGAATTCGTC-GTNATYGGNCAYGTGAYTC-3' from exon 2 and antisense: 5'-GCGAATTCGARTCCATCTTRTTNACNCCNCA-3' from exon 4); (abbreviations according to the IUPAC code). At the 5'-ends of both primers, the *Eco*RI cleavage sites were designated for the cloning of the

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Abbreviation: RT-PCR, reverse transcription polymerase chain reaction. SSC, standard saline citrate. MMLV-RT, moloney murine leukemia virus reverse transcriptase.

product of following polymerase chain reaction (PCR) into plasmid vector.

Single-stranded cDNA was synthesized from 1 µg of total RNA extracted from medaka liver with 200 units of MMLV-RT in 20 µl of a reaction mixture containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 0.1 mg/ml bovine serum albumin (BSA), 1 mM each dNTP, 5 µM random hexamer, and 20 units of ribonuclease inhibitor RNasin (TOYOBO). A quarter of the resultant cDNA was used as a template in the PCR, which was carried out in a 25 µl solution containing 8 units of Taq DNA polymerase (TAKARA), 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM MgCl₂, 0.1 mg/ml BSA, 0.2 mM each dNTP. The con-

dition of PCR was 90 s at 94°C, 2 min at 50°C, and 2.5 min at 72°C for 30 cycles (ASTEC, Program Temp. Control System PC-700).

The product (about 450 bp) of PCR was cloned into pBluescript II KS+ and sequenced by a PCR procedure employing fluorescent dideoxynucleotides and model 373A automated DNA sequencer (Applied Biosystems). The *Eco*RI fragment of the clone presumed to a counterpart of mammalian EF-1α was used as a probe for cDNA library screening.

Construction and Screening of a cDNA Library

Total RNA was isolated from 0.1 g of medaka liver by

-51	TAGGGGCTGCAGCTTTGGCCGTGAAAACCCAGAAACACCGAAACATACAAA	-1
1	M G K E K I H I N I V V I G H V D S G K S T S T G H L I Y K	30
1	ATGGGAAAGGAAAAATCCACATTAACATCGTGGTCATTGGCCATGTCGACTCCGGCAAGTCCACCTCCACCGGTACCTGATCTACAAG	90
31	C G G I D K R T I E K F E K E A A E M G K G S F K Y A W V L	60
91	TGCGGAGGAATCGACAAAGAACCATCGAAAAGTTCGAGAAGGAAGCCGCTGAGATGGGCAAGGGCTCCTTCAAGTACGCTGGGTGTG	180
61	D K L K A E R E R G I T I D I A L W K F E T S K Y Y V T I I	90
181	GACAACTCAAGGCTGAGCGTGAGCGTGGTATCACCATCGACATCGCTCTGTGGAAGTTTGAGACCAGCAAGTACTATGTGACCATCATT	270
91	D A P G H R D F I K N M I T G T S Q A D C A V L I V A A G V	120
271	GATGCCCCCTGGACACAGACTTCATCAAGAACATGATCACTGGAACCTCCAGGCTGACTGTGCTGTGCTGATCGTGGCTGCTGGCGTT	360
121	G E F E A G I S K N G Q T R E H A L L A F T L G V K Q L I V	150
361	GGTGAGTTTGAGGCTGGTATCTCAAAGAACGGGACAGCCGCTGAGCACGCCCTGCTGGCCTTCACTCTTGAGAGTGAAGCAGCTCATCGTT	450
151	G V N K M D S T E P P Y S Q A R F E E I Q K E V S T Y I K K	180
451	GGCGTCAACAGATGGACTCCACCGAGCCCCCTTACAGCCAGGCCCCGTTTGGAGAAATCCAAAGGAAGTAAGCACCTACATCAAGAAG	540
181	I G Y N P A A V A F V P I S G W H G D N M L E A S D K M S W	210
541	ATCGGCTACAACCCAGCTGCAAGTGGCTTTTGTCCCATCTCTGGATGGCAGGAGACAACATGCTGGAGGCCAGCGACAAGATGAGCTGG	630
211	F K G W K I E R K D G N A S G T T L L E A L D A I L P P S R	240
631	TTCAAGGGCTGGAAGATTGAGCGCAAGGACGGAATGCCAGTGGAAACAACCTCTGAAGCTCTTGATGCTATCCTGCCACCTTCTCGC	720
241	P T D K P L R L P L Q D V Y K I G G I G T V P V G R V E T G	270
721	CCCACCGACAAACCCCTGCGTCTGCCCTGCAAGGACGCTCTACAAATCGCGGTATTGGAACGTGCCCTGTGCGGCGTGTGAGACTGGA	810
271	V L K P G M V V T F A P P N L T T E V K S V E M H H E S L P	300
811	GTCCTGAAGCCAGGATGTTGTACCTTTGCTCCCCCAACCTGACCACTGAAGTCAAGTCAGTGAGATGCACCACGAGTCTTTACCA	900
301	E A V P G D N V G F N I K N V S V K E I R R G Y V A G D S K	330
901	GAGCTGTGCGCGGTGACACGTTGGCTTCAACATCAAGAACGTGTCCGTCAAGAAATCCGTCTGGATACGTGCTGGTGACAGCAAG	990
331	N D P P K A A A S F N A Q V I I L N H P G Q I N Q G Y A P V	360
991	AACGACCACCAAAGGCTGCTGCCAGCTTCAACGCTCAGGTATCATCTCTGAACCAACCCAGGCCAGATCAACAGGGCTATGCCCTGTG	1080
361	L D C H T A H I A C K F N E L I E K I D R R S G K K L E D N	390
1081	CTGGACTGCCACACTGCTCACATCGCTGCAAGTTCACGAGCTCATTGAGAAGATCGACCGTCTGTTCTGGCAAGAACTGGAAGACAAC	1170
391	P K F V K S G D A A I V K L I P Q K P M V V E P F S N Y P P	420
1171	CCCAAGTTTGTTAAGCTGGAGATGCTGCCATTGTCAAGCTGATCCACAGAAGCCCATGGTTGTGGAGCCCTTCTCAAACTACCTCTCT	1260
421	L G R F A V R D M R Q T V A V G V I K A V D T K E I S G K T	450
1261	CTTGAGCGTTTGTGCTGCGTGACATGAGGCAGACCGTGGCTGTTGGTGTCAAGGCTGTTGATACCAAGGAAATTTCCGGCAAGACG	1350
451	T K A A E K A Q K K K *	462
1351	ACCAAGGCTGCAGAGAAAGCCAGAGAAAGAAATGAATGGTCTGTCAGGATGCCAAACAAGACGTGTGCCGGCAGCAGCAGGCAACTGCC	1380
1441	TCCATGCATCTTCTCCATCCTGGGTATTACTCTGAGGCATAGTTCTATTCTGAAGGACTGGCTTATGCTGATCAAAACCCATCGC	1530
1531	AAAAGTTTTTCGAGGAAAAAGAAACGTGGCATAGTCATTTAACTAAATCACAGGACGGTGCCTCTTTAAGTTTGAATTTAAACTGTTTC	1620
1620	ATTGTTCAATGCCACAAGTTGATTGGAACAGAAGCTGCTGGAATTAATAAATAAATGATTTGAAAAATAAAAAAAAAA	

Fig. 1. The nucleotide sequence of the cDNA encoding medaka EF1α and deduced amino acid sequence. The start (ATG), stop (TGA), and polyadenylation signal sequences are underlined. The nucleotide sequence has been assigned DDBJ, EMBL, and GenBank accession No. AB013606.

pared by use of the Megaprime DNA labeling system (Promega). After hybridization, the filters were washed once with $2\times$ SSC ($1\times$ SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) at room temperature and two times with $2\times$ SSC at 65°C prior to autoradiography.

Northern Blot Analysis

Total RNA was isolated from liver, muscle, eye, gill, tail fin, and brain of medaka as described above. Five micrograms of total RNA from each tissue was subjected to

Northern blot analysis. The probe and the condition of hybridization and washing were the same as those of cDNA library screening.

Results and Discussion

Cloning of Medaka *EF1 α*

Four positive clones were isolated from the medaka liver cDNA library, and one clone contained about 1.6 kbp DNA (Fig. 1). The 1383 bp DNA from position 1 to 1383 was identified as an *EF1 α* -encoding open reading frame (ORF) because of its similarity to a number of eukaryotic *EF1 α* ORF in the DDBJ database (Fig. 1). At the DNA sequence level the coding region is 85% identical to zebrafish and 80% identical to human cDNAs, respectively. As for *Xenopus laevis* two isoforms of *EF1 α* were reported; e*EF1 α* O is an oocyte form and e*EF1 α* S is a somite form 8). Nordnes *et al.*, mentioned that the zebrafish *EF1 α* is a somite form because of higher identity to somite form (92%) than oocyte form (90%).¹⁷⁾ On the other hand, medaka *EF1 α* showed almost the same identity to both *Xenopus laevis* *EF1 α* S (74.7% to e*EF1 α* S and 74.5% to e*EF1 α* O).

Multiple alignment of deduced amino acid sequence indicates 90%, 87%, 87%, 88%, and 88% identities between medaka and zebrafish, *Xenopus laevis* *EF1 α* O, *Xenopus laevis* *EF1 α* S, chicken, and rat, respectively by malige program in DDBJ (Fig. 2). Especially, the putative effector domain interacting with the ribosome¹⁷⁾ (at the position from 51 to 72) is identical in all vertebrate and the four GTP binding domains¹⁰⁾ (at the position from 12 to 21, from 88 to 100, from 148 to 156, and from 286 to 300) were well conserved (Fig. 2). *EF1 α* promotes the GTP-dependent binding of aminoacyl-tRNA into the acceptor site of elongating ribosomes during mRNA translation.¹⁾ These findings indicate the importance of these domains on the function of *EF1 α* . Though the meaning is not clear, *EF1 α* is known to be involved in variety of cellular processes besides protein synthesis.²⁻⁴⁾ One of the additional functions of *EF1 α* is to bind actin.²⁾ A putative β -actin-binding domain²⁾ (at the position from 161 to 180) was also well conserved in medaka *EF1 α* (Fig. 2). In the latter half part of medaka *EF1 α* , 15 of 17 unique amino acid changes were observed. The most unique feature of medaka *EF1 α* is one amino acid deletion at the position 447.

Expression of *EF1 α* Gene

The expression of *EF1 α* gene among tissues was investigated by Northern blot analysis (Fig. 3). A single hybridized signal of about 1.8 kb, which agreed well with the sizes of zebrafish¹⁴⁾ (1.7 kb), chicken¹⁵⁾ (1.8 kb), and rat¹²⁾ (1.8 and 2.0 kb), was observed in all tissues tested such as, liver, muscle, eye, gill, tail fin, and brain (Fig. 3). This ubiquitous expression pattern suggests the importance of *EF1 α* in all tissues. This fact also suggests that the *EF1 α* mRNA is useful as a internal standard on Northern blot analysis.

EF1 α is concerned with polypeptide elongation and essential for living cells. 1) As for human,^{5,13)} mouse,¹²⁾ rat,^{10,12)} *Xenopus laevis*,^{8,9)} and *Drosophila melanogaster*,⁷⁾ there are several genes encoding *EF1 α* and the expression of these genes depends on developmental stage and/or cell (tissue) differentiation. However, it was reported that

Fig. 3. Expression of *EF1 α* in medaka tissues. Lower panel: Five micrograms of total RNA were subjected to the Northern blot analysis. Details are described in "Materials and Methods". L, liver; G, gill; E, eye; K, kidney; B, brain; F, tail fin; T, testis; O, ovary; M, muscle. Upper panel: Total RNA in agarose gel was stained by ethidium bromide.

there is only one gene encoding *EF1 α* in zebrafish.¹⁴⁾ At present, it is not clear whether single gene structure of *EF1 α* observed in zebrafish is common among fish species or not. To clarify this point, the further studies on the gene structure of *EF1 α* in medaka chromosome, together with the nucleotide sequence defined in this study, are in progress.

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