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Molecular Cloning and Characterization of a Heat Shock Protein 70-related cDNA from Olive Flounder (*Paralichthys olivaceus*)

Woo Jin Kim, Jeong Ho Lee, Kyung Kil Kim, Sang Jun Lee, Ho Sung Kang* and Han Do Kim*

Biotechnology Division, National Fisheries Research and Development Institute, Pusan 626-900, Korea
**Department of Molecular Biology, Pusan National University, Pusan 609-735, Korea*

The complete nucleotide sequence of olive flounder (*Paralichthys olivaceus*) *hsp70*-related cDNA was determined by RT- and RACE-PCR methods. A full-length of *hsp70*-related cDNA has an open reading frame of 1.95 kb encoding 650 amino acids with a calculated molecular weight of 71.1 kD. A corresponding hsp70-related protein contains a number of conserved elements including an ATP-binding domain, a nuclear localization signal and the carboxyl terminal motif, EEVD, which may have a role in chaperone function. Comparison of nucleotide and predicted amino acid sequence between olive flounder *hsp70*-related gene and *hsp/hsc70* genes of other species revealed a high similarity with the cognate form of these genes. These results indicated that we recovered likely to be a olive flounder cognate *hsc70* gene.

Key words : Hsp/hsc70 gene, Olive flounder, *Paralichthys olivaceus*, RT- and RACE-PCR

Introduction

In all cells and organisms examined so far, heat shock proteins (hsps) are synthesized in response to an increase of temperature above the normal physiological level, and also by physiological stresses such as amino acid analogues, heavy metals, and sodium arsenite (Kothary and Candido, 1982 ; Misra et al., 1989). Although a limited number of species have been examined, the fish hsps can be grouped into six families based upon their molecular masses: 100 kD (hsp100), 84-95 kD (hsp90), 65-76 kD (hsp70), 59-62 kD (hsp60), 39-42 kD (hsp40), and 27-30 kD (hsp27) (Gedamu et al., 1983 ; Mooser et al., 1986). Among the different classes of hsps, the most abundant hsp is a hsp70 which is remarkably conserved in organisms from bacteria to man (Schlesinger et al., 1982). In higher organisms, *hsp70* is a multigenic family including the transiently stress-inducible form of hsp70 proteins involved in the cellular response to various types of stress, and one or more cognate protein(s) (hsc70) are constitutively expressed under normal growth conditions (Lindquist and Craig, 1988).

The *hsp70*-related genes, first identified in *D. melaganoster*, from many different organisms have been isolated and characterized (Holmgren et al., 1979 ; Ingolia et al., 1980 ; Bienz, 1984 ; Hunt and Morimoto, 1985 ; Luft et al., 1996), but they are rarely characterized in marine fish. Therefore, the aim of this study is to isolate and sequence the olive flounder (*Paralichthys olivaceus*) *hsp70*-related cDNA. Gene cloning and sequencing studies on hsp70/hsc70 show that the primary structure of these proteins has highly conserved during evolution in species ranging from bacteria to man.

Many investigators have suggested that besides their function as molecular chaperone in protein assembly and transport (Ellis and Van der Vies, 1991), hsc70 might be involved in embryonic development (Bienz, 1984 ; Santacruz et al., 1997). However, the exact function of this hsc70 in the embryonic development is not clearly understood. The physiological role of hsc70 in oocytes may be important for growth and maturation of oocytes and for early embryonic development. In the present study, we amplified a cDNA fragment corresponding to the conservative regions of olive flounder hs70-related protein by reverse transcription-polymerase chain reaction (RT-PCR). The complete cDNA sequence for this protein was then obtain by 3′- and 5′- rapid amplification of cDNA ends (RACE) method.

Materials and Methods

Fish

The olive flounder were obtained from Koje Hatchery of National Fisheries Research and Development Institute and maintained in 6 tons flow-through tank at 18±1℃ under a natural photoperiod.

RNA preparation

Total RNA was isolated from olive flounder hepatocytes using Trizol RNA isolation reagent (GIBCO/BRL, MD, USA) according to instructions of the manufacturer based on the acid guanidium thiocyanate-phenol-chloroform extraction method (Chomczynski et al., 1987).

RT-PCR and 3- and 5- RACE amplification

The complete cDNA sequence encoding hsp 70-related protein was determined by the three step strategy, (i) RT-PCR of the specific region for hsp70-related protein, (ii) 3′-RACE, and (iii) 5′-RACE. Table 1 summarizes primers used for cDNA synthesis and amplification of olive flounder *hsp70*-related cDNA by RT- and RACE-RCR. Synthetic oligonucleotides designed accor-

Table 1. Primers used for cDNA synthesis and amplification of *hsp70*-related cDNA

RT- and RACE-PCR or cDNA synthesis	Primers	Location (primer)
Hsp70-related cDNA fragment		
cDNA synthesis	Oligo (dT)	
RT-PCR	RT1 + RT2	109-129 (RT1) 2017-2037 (RT2)
Full-length *hsp70*-related cDNA		
cDNA synthesis	Oligo (dT)	
RT-RCR	RT3 + RT4	1-27 (RT3) 2223-2250 (RT4)
3′-RACE		
cDNA synthesis	Oligo (dT)-adaptor	
First-round PCR	Adaptor + RA1	1483-1503 (RA1)
Second-round PCR	Adaptor + RA2	1906-1926 (RA2)
5′-RACE		
cDNA synthesis	RT2	2017-2037 (RT2)
First-round PCR	AAP + RA3	682-702 (RA3)
Second-round PCR	AUAP + RA4	130-152 (RA4)

ding to rainbow trout *hsc70* cDNA (Zafarullah et al., 1992) were used for RT-PCR amplification of cDNA prepared from total RNA of olive flounder hepatocytes by oligo (dT)-primed reverse transcription. The sense primer RT1 5′-GGCATCGATCTCGGGACCACC-3′ corresponded to nucleotides 121-141 and the antisense primer RT2 5′-GTCGACTTCCTCAATGGTTGG-3′ corresponded to nucleotides 1933-1953 of the rainbow trout sequence. These primers were derived from highly conserved amino acids of hsp70-related proteins in many organisms (Ingolia et al., 1980 ; Hunt and Morimoto, 1985 ; Zafarullah et al., 1992 ; Santacruz et al., 1997). The PCR cycle consisted of 1 min denaturation at 94℃, 1 min annealing at 55℃, and 1 min extension at 72℃ for 35 cycles. Expected PCR products were cloned into the TA cloning vector pCR2.1 (Invitrogen, San Diego, CA) and sequenced. The 3′- and 5′- RACE was carried out as described by Frohman *et al* (1990). 3′- and 5′-end gene specific primers were designed based on the sequence which had been determined from clones obtained by the above RT-PCR. For the 3′-RACE, the total RNA was reverse- transcribed using oligo(dT)-containing adaptor primer (AP) 5′-GGCCACGCGTCGACT- AGTAC(T)$_{17}$-3′. The synthesized cDNA was subjected to the first round PCR amplification using the AP as antisense primer and the primer RA1 5′-CTTGC-TCCT- CGTGGTGTTCCC-3′ as a sense primer. The second round PCR amplification was carried out using the AP and the primer RA2 5′-ATCACTAAGCTGTACCAGAGTG-3′. For the 5′-RACE, the total RNA was reverse-transcribed into single-stranded cDNA using the primer RT2 followed by poly(dC) addition to the 3′ end of the single-stranded cDNA. The synthesized cDNA was amplified by the first round PCR using abridged anchor primer (AAP) 5′-GGCC-ACGCGTCGACTAGTACGGGIIGGGIIGGGIIG-3′ (where I = inosine) as a sense primer and the primer RA3 5′-AAAGGTGCCACCACCAAG AT-C-3′ as an antisense primer. The second round PCR was performed using abridged universal amplification primer (AUAP) 5′-GGCCACGCG-TCGACTAGTAC-3′ and the primer RA4 5′-TGGAACACACCAACACAGGAGTA-3′. To clone the full-length cDNA, the primers were designed based on the sequence which had been determined from clones obtained by the above 3′- and 5′-RACE. The total RNA was converted into single-stranded cDNA using oligo(dT) as described above. RT-PCR was performed using the sense primer RT3 5′-TGCCCTTTCTTTC-TCAAACCGGCAAGG-3′ and the antisense primer RT4 5′-GAGGTTTCATGTTTATTGACAA-GGCAG-3′.

DNA sequencing and computer analysis of predicted amino acid sequences

cDNA was sequenced with a DNA sequencer (model 373A) using DyeDeoxy Terminator Cycle Sequencing kits (Applied Biosystems, Weiterstadt, Germany) according to the manufacturers instructions. Nucleotide sequences were analyzed by the DNASIS 2.5 program (Hitachi Software, Tokyo, Japan). Sequence comparisons were done using the multiple alignment program Clustal V (EMBL, Heidelberg, Germany).

Results and Discussion

Isolation and characterization of the olive flounder hsp70-related cDNA

To isolate olive flounder *hsp70*-related cDNA fragment, we performed RT-PCR using a single pair of primers, RT1 and RT2. An expected

1,929 bp cDNA fragment (Fig. 1) was cloned into the TA-vector and sequenced. The nucleotide sequence of the 1,929 bp fragment revealed significant homologies to known *hsp/hsc70* genes (data not shown). The remaining sequence of 3'- and 5'- end of olive flounder *hsp70*-related cDNA was determined by the RACE procedure. The first (primers, AP and RA1) and second round PCR (primers, AP and RA2) for the 3'-end of *hsp70*-related cDNA produced distinct bands of approximately 788 bp and 365 bp size, respectively, on agarose gel electrophoresis (Fig. 2A). After isolation and cloning of 365 bp products, the nucleotide sequence encoding 3'-end of *hsp70*-related cDNA were identified by sequence analysis, indicating that this product contained open reading frame for the C-terminal portion and 3'untranslated region (UTR). Analysis of the 5'-RACE products

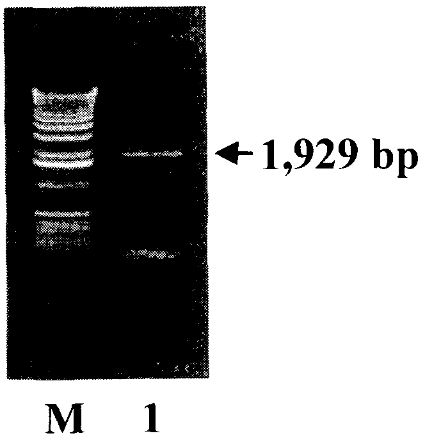

Fig. 1. Amplification of olive flounder *hsp70*-related cDNA fragment by RT-PCR. RT-PCR was performed with cDNA prepared total RNA of olive flounder hepatocytes using two primers, RT1 and RT2. The amplified products (lane 1) of 1,929 bp were electrophoresed in 1% agarose gel. Lane M indicates the 1 kb ladder DNA molecular weight marker. Arrow indicates the amplified products.

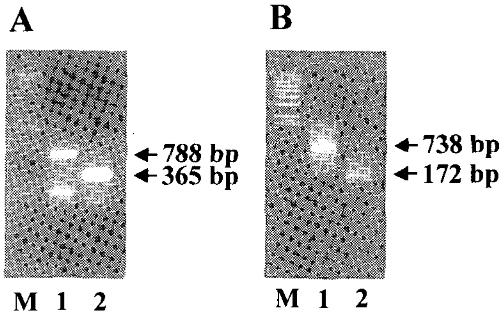

Fig. 2. Amplification of olive flounder *hsp70*-related cDNA ends by RACE-PCR. For the 3'-RACE, the synthesized cDNA was subjected to the first round PCR using the AP and RA1 primers (panel A, lane 1) and the second round PCR using AP and RA2 primers (panel A, lane 2). For the 5'-RACE, the synthesized cDNA was amplified by the first round PCR using AAP and RA3 primers (panel B, lane 1) and second round PCR using AUAP and RA4 primers (panel B, lane 2). The RACE-PCR products were electrophoresed in 1% agarose gel. Lane M indicates the 1 kb ladder DNA molecular weight marker. Arrow indicates the amplified products.

by agarose gel electrophoresis revealed one major band of about 738 bp for first round PCR (primers, AAP and RA3) and 172 bp for second round PCR (primers, AUAP and RA4) (Fig. 2B). Sequence analysis of 172 bp fragment showed that this fragment contained open reading frame for the N-terminal portion and 5'UTR. Nucleotide sequences of 3'- and 5'-end cDNA was identical in the regions of overlapping with the sequence of the *hsp70*-related cDNA fragment. To minimize mistakes introduced by Taq DNA polymerase, at least four independent clones from RT- and RACE-PCR were sequenced. To clone the full-length of *hsp70*-related cDNA, RT-PCR was also carried out using primers (RT3 and RT4) based on the nucleotide sequence information from 3'- and 5'-RACE. The size of the amplified product was approximately 2,250 bp (Fig. 3) and the products were cloned into the TA-vector and sequenced. The nucleotide sequences of the overlapping portion were also identical to the sequence of the clones obtained by the RT- and RACE-PCR, suggesting that we had obtained the complete *hsp70*-related cDNA. The 2,250 bp nucleotide sequence contained the entire open reading frame of 1950bp encoding 650 amino acid residues, the 5'UTR and the 3'UTR with polyadenylation site (AATAAA) (Fig. 4). The calculated molecular mass of this olive flounder *hsp70*-related cDNA product corresponds to about 71.1 kD.

The olive flounder *hsp70*-related protein exhibits three evolutionary conserved domains typical for hsp/hsc70 family (Fig. 4). These three domains, called signature 1 (IDLGTTYS, amino acids 9-16), signature 2 (DLGGGTFD), amino acids 199-206), and signature 3 (EEVD, amino acids 647-650), are known to be conserved in most hsp70/hsc70 proteins (Gupta and Golding, 1993). The recent finding that the EEVD sequence of human hsp70 is necessary for several chaperone functions (Freeman et al., 1995) suggests that olive flounder hsp70-related protein may also act as chaperones. It also contains a potential nuclear location signal (amino acids 246-262) (Fig. 4), which has been described in mammalian hsc70 (Dang and Lee, 1989). This motif would be necessary to the nuclear targeting (Shulga et al., 1996). In addition, the tetrapeptide repeat sequence (GGMP, amino acids 615-622) is present in C-terminal portion. This sequence, which is present in hsp/hsc70 family and hsp60 family, may be an important site for the folding/unfolding function (Bosch et al., 1991).

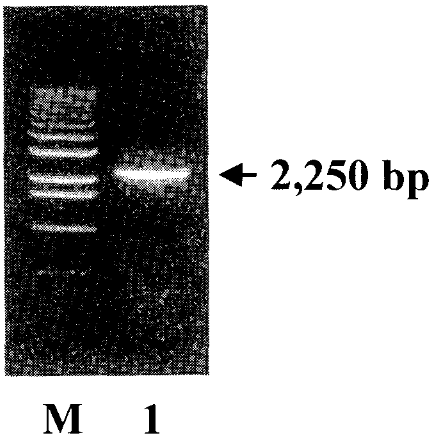

Fig. 3. Amplification of full-length olive flounder *hsp70*-related cDNA by RT-PCR. RT-PCR was performed with cDNA prepared total RNA of olive flounder hepatocytes using two primers, RT3 and RT4. The amplified products (lane 1) of 2,250 bp were electrophoresed in 1% agarose gel. Lane M indicates the 1 kb ladder DNA molecular weight marker. Arrow indicates the amplified products.

Analysis of the predicted amino acid sequences of olive flounder hsp70-related cDNA

The predicted amino acid sequence of olive flounder *hsp70*-related cDNA was compared with the previously published sequences for rainbow trout hsc70 (Zafarullah, et al., 1992), zebrafish hsc70 (Santacruz et al., 1997), human hsc70 (Dworniczak and Mirault, 1987), human hsp70 (Hunt and Morimoto, 1985), mouse hsp70 (Hunt and Calderwood, 1990), and *Drosophila* hsp70 (Ingolia et al., 1980) (Fig. 5). It was found to be highly homologous to this hsp/hsc70 family. Although these comparisons show a high degree of conservation in the N-terminal portions, their C-terminal portions diverse significantly. This observation suggests that the C-terminal domain is species-specific (Bienz, 1984). Hsp/hsc70 family can also be divided into two functional domains corresponding to a

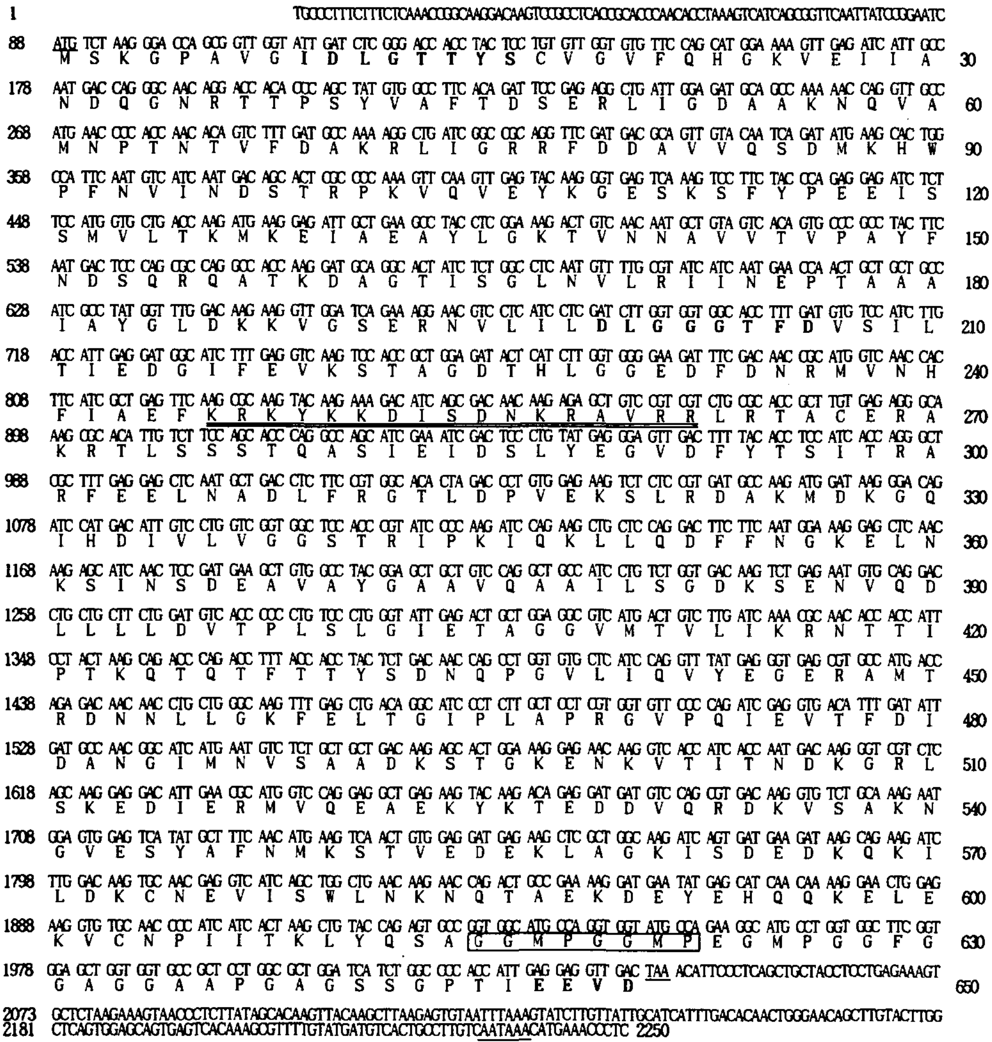

Fig. 4. The nucleotide and predicted amino acid sequence of olive flounder *hsp70*-related cDNA. The nucleotide sequence data has been submitted to the EMBL/GenBank Data Libraries with accession number AF053059. The nucleotide number is shown on the left and amino acid residue number is shown on the right. The translational start codon (ATG), stop codon (TAA) and putative polyadenylation signal (AATAAA) in the 3′ UTR are underlined. Three conserved elements (signature 1, IDLGTTYS; signature 2, DLGGGTFD; signature 3, EEVD) are bolded. Putative nuclear localization signal is double underlined. The tetrapeptide repeat sequence (GGMP) is boxed.

region necessary for nucleotide biding and a distinct region required for nucleolar localization and peptide binding (Milarski and Morimoto, 1989). Indeed, the ATP-binding domain of olive flounder hsp70-related protein (amino acids 122-264) revealed a high Similarity with hsc70

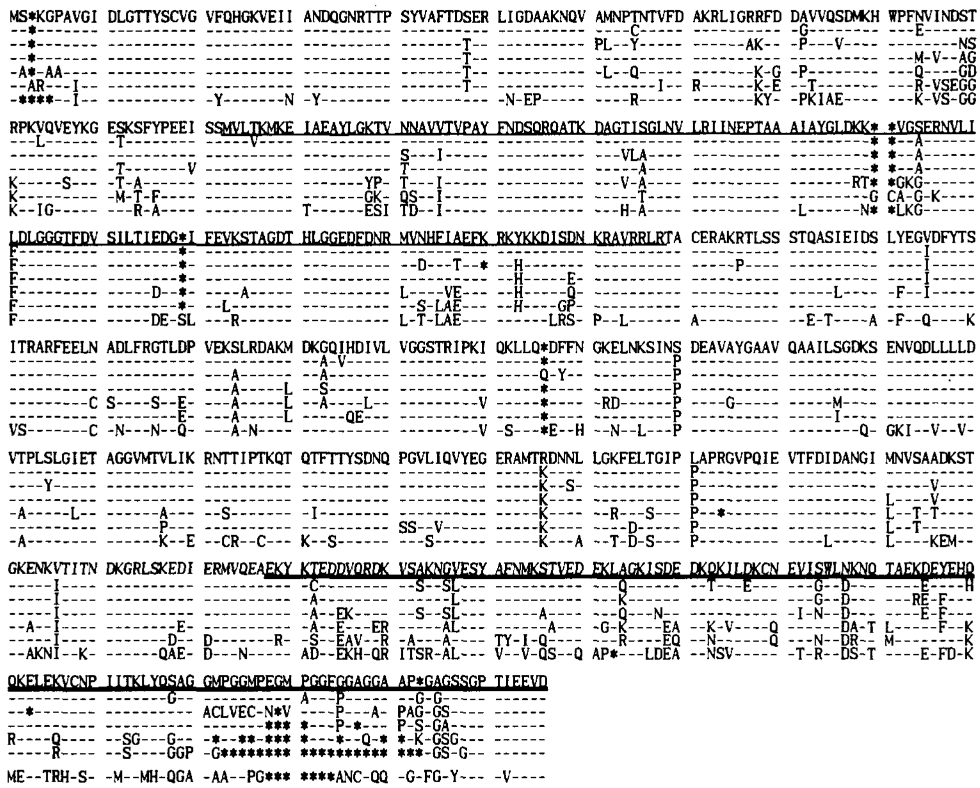

Fig. 5. Comparison of the predicted amino acid sequence of olive flounder hsp70-related protein (a) with rainbow trout hsc70 (b), zebrafish hsc70 (c), human hsc70 (d), human hsp70 (e), mouse hsp70 (f), and *Drosophila* hsp70 (g). The dashes (-) indicate identical amino acid residues and squares (*) denote a gap or deletion in the sequence. The ATP-binding domain is underlined, whereas the peptide-binding domain is double underlined.

of other species: 97.2% Similarity with rainbow trout hsc70, 92.3% with zebrafish hsc70, and 95.1% with human hsc70. However, it represents only 85.9% Similarity with human hsp70, 85.2% identity with mouse hsp70, and 78.2% Similarity with *Drosophila* hsp70 (data not shown). Moreover, at the carboxyl-end of olive flounder hsp70-related protein (amino acids 523-650), the similarity of the peptide- binding domain was also higher with the constitutive hsc70 than with the inducible hsp70, but at a lower degree similarity with rainbow trout hsc70 was 87.5%, 81.3% with human hsc70, only 68.8% with human hsp70, and only 59.4% with mouse hsp70 (data not shown). As previously described by other investigators, these domains were more divergent in the same species than between different species (Ali et al., 1996).

We found that the entire predicted amino acid sequence of olive flounder hsp70-related protein has a similarity of 95.1% with rainbow trout hsc70 (Zafarullah et al., 1992), 91.0% with zebrafish hsc70 (Santacruz et al., 1997), 92.6% with human hsc70 (Dworniczak and Mirault,

Table 2. Nucleotide and amino acid similarity between olive flounder hsp70-related protein and hsp and/or hsc70 protein of other species

Species	Hsp/hsc70	Similarity with olive flounder hsc70-related protein	
		Amino acid (%)	Nucleotide* (%)
Rainbow trout	hsc71	95.1	85.9
Mouse	hsc70	93.0	82.1
	hsp70.1	83.2	74.3
Human	hsc71	92.6	80.5
	hsp70	83.3	75.0
Zebrafish	hsc70	91.0	82.4
Drosophila	hsc70.4	84.6	76.2
	hsp70.1	71.4	67.4

The percentage similarity of amino acid sequence was calculated using the multiple alignment program Clustal V.
*Only the nucletide sequence in translated region were compared with each other.

1987), 93% with mouse hsc70 (Giebel et al., 1988), and 84. 6% *Drosophila* hsc70 (Perkins et al., 1990) (Table 2). However, it shows only 83.3%, 83.2%, 71.4% similarity with the heat-inducible form of human, mouse, and *Drosophila* hsp70, respectively (Table 2). When nucleotide sequence in translated region of olive flounder hsp70-related protein was compared with *hsp/hsc70* genes of other species, this gene has a similarity of 85.9% with rainbow trout *hsc70*, 82.4% with zebrafish *hsc70*, 80.5% with human *hsc70*, 82.1% with mouse *hsc70*, 76.2% *Drosophila hsc70*, 75.0% with human *hsp70*, 74.3% with mouse *hsp70*, and 67.4% *Drosophila hsp70* (Table 2). The observed high similarity of the nucleotide and amino acid with constitutive *hsc70* of other species provides evidence that we recovered likely to be a olive flounder cognate *hsc70* gene.

The isolation of this gene from the olive flounder opens the door to study the role of constitutively produced members of the hsp70 gene family in marine fish. Many investigators (Billoud et al., 1993 ; Santacruz et al., 1997) have suggested that besides their function as molecular chaperone, hsc70 might be involved in oogenesis and embryogenesis. Therefore, we will further investigate the expression and specific role of olive flounder hsp70-related protein during oogenesis and embryogenesis.

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References

Ali, A., L. Salter-Cid, M. F. Flajnik and J. J. Heikkila, 1996. Isolation and characterization of a cDNA encoding a *Xenopus* 70-kDa heat shock cognate protein, Hsc70. I. Comp. Biochem. Physiol. B, 113 : 681-687.

Bienz, M, 1984. *Xenopus hsp70* genes are constitutively expressed in injected oocytes. EMBO J., 3 : 2477-2483.

Billoud, B., M. L. Rodriguez-Martin, L. Bérard, N. Moreau and N. Angelier, 1993. Constitutive expression of a somatic heat-inducible hsp70 gene during amphibian oogenesis. Development, 119 : 921-932.

Bosch, T. C. G., K. Gellner and G. Praetzel. 1991. The stress response in the freshwater polyp hydra. p. 133-142. Heat Shock. (B. Maresca and S. Lindquist eds.). Springer-Verlag, Heidelberg.

Chomczynski, P. and N. Sacchi, 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., 162 : 156-159.

Dang, C. V. and W. M. Lee, 1989. Nuclear and nucleolar targeting sequences of c-erb-A, c-myb, N-myc, p53, HSP70, and HIV tat proteins. J. Biol. Chem., 264 : 18019-18023.

Dworniczak, B. and M. E. Mirault, 1987. Structure and expression of a human gene coding for a 71 kd heat shock cognate protein. Nucleic Acids Res., 15 : 5181-5197.

Ellis, R. J. and S. M. Van der Vies, 1991. Molecular chaperones. Ann. Rev. Biochem., 60 : 321-347.

Freeman, B. C., M. P. Myers, R. Schumacher and R. I. Morimoto, 1995. Identification of a regulatory motif in hsp70 that affects ATPase activity, substrate binding and interaction with HDJ-1. EMBO J., 14 : 2281-2292.

Frohman, M. A, 1990. RACE: rapid amplification of cDNA ends. p. 28-38. PCR protocol: A Guide to Methods and Applications (M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White eds.). Academic Press, San Diego, CA.

Gedamu, L., B. Culham and J. J. Heikkila, 1983. Analysis of the temperature-dependent temporal pattern of heat-shock protein synthesis in fish cells. Biosci. Rep., 3 : 647-658.

Giebel, L. B., B. P. Dworniczak and E. K. F. Bautz, 1988. Developmental regulation of a constitutively expressed mouse mRNA encoding a 72-kDa heat shock-like protein. Dev. Biol., 125 : 200-207.

Gupta, R. S, and G. B. Golding, 1993. Evolution of HSP70 gene and its implications regarding relationships between archaebacteria, eubacteria, and eukaryotes. J. Mol. Evol., 37 : 573-582.

Holmgren, R., K. Livak, R. Morimoto, R. Freund and M. Meselson, 1979. Studies of cloned sequences from four *Drosophila* heat shock loci. Cell, 18 : 1359-1370.

Hunt, C. and S. Calderwood, 1990. Characterization and sequence of a mouse *hsp70* gene and its expression in mouse cell lines. Gene, 87 : 199-204.

Hunt, C. and R. I. Morimoto, 1985. Conserved features of eukaryotic hsp70 genes revealed by comparison with the nucleotide sequence of human hsp70. Proc. Natl. Acad. Sci. USA, 82 : 6455-6459.

Ingolia, T. D., E .A. Craig and B. J. McCarthy, 1980. Sequence of three copies of the gene for the major *Drosophila* heat shock induced protein and their flanking regions. Cell, 21 : 669-679.

Kothary, R. K. and E. P. M. Candido, 1982. Induction of a novel set of polypeptides by heat shock or sodium arsenite in cultured cells of rainbow trout, *Salmo gairdnerii*. Can. J. Biochem., 60 : 347-355.

Lindquist, S. and E. A. Craig, 1988. The heak-shock proteins. Annu. Rev. Genet., 22 : 631-677.

Luft, J. C., M. R. Wilson, J. E. Bly, N. W. Miller and L. W. Clem, 1996. Identification and characterization of a heat shock protein 70 family member in channel catfish (*Ictalurus punctatus*). Comp. Biochem. Physiol. B, 113 : 169-174.

Milarski, K. L. and R. I. Morimoto, 1989. Mutational analysis of the human HSP70 protein: distinct domains for nucleolar localization and adenosine triphosphate binding. J. Cell. Biol., 109 : 1947-1962.

Misra, S., M. Zafarullah, J. Price-Haughey and L. Gedamu, 1989. Analysis of stress- induced gene expression in fish cell lines exposed to heavy metals and heat shock. Biochim. Biophys. Acta, 1007 : 325-333.

Mooser, D. D., J. J. Heikkila and N. C. Bols, 1986. Temperature ranges over which rainbow trout fibroblasts survive and synthesize heat-shock proteins. J. Cell. Physiol., 128 : 432-440.

Perkins, L. A., J. S. Doctor, K. Zhang, L. Stinson, N. Perrimon and E. A. Craig, 1990. Molecular and developmental characterization of the heat shock cognate 4 gene of *Drosophila melanogaster*. Mol. Cell. Biol., 10 : 3232-3238.

Santacruz, H., S. Vriz and N. Angelier, 1997.

Molecular characterization of a heat shock cognate cDNA of zebrafish and developmental expression of the corresponding transcripts. Dev. Genet., 21 : 223-233.

Schlesinger, M. J., M. Ashburner and A. Tissieres, 1982. Heat Shock. In From Bacteria to Man. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 440pp.

Shulga, N., P. Roberts, Z. Gu, M. M. Tabb, M. Nomura and D. S. Goldfarb, 1996. In vivo nuclear transport kinetics in *Saccharomyces cerevisiae*: a role for heat shock rotein 7 during targeting and translocation. J. Cell. Biol., 135 : 329-339.

Zafarullah, M., J. Wisniewski, N. W. Shworak, S. Schieman, S. Misra and L. Gedamu, 1992. Molecular cloning and characterization of a constitutively expressed heat- shock-cognate hsc71 gene from rainbow trout. Eur. J. Biochem., 204 : 893-900.