cDNA Cloning of Heat-inducible HSP70, a 70.6 kDa Heat Shock Protein, in Japanese Flounder *Paralichthys olivaceus*

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Full-length cDNA for a 70.6 kDa heat-inducible heat shock protein (HSP70), a member of the HSP70 family, was isolated from a cDNA library of cultured cells in early passage originated in Japanese flounder embryos (JFE cells). It has a single ORF of 1920 bp that encodes a protein of 70.6 kDa. Japanese flounder HSP70 contains an EEVD peptide motif at C-terminal end which is a common feature of the cytosolic HSP70 family. Japanese flounder HSP70 is 86.0%, 83.8%, and 83.6% identical in primary structure to chinook salmon HSP70, bovine HSP70, and human HSP70, respectively. Japanese flounder HSP70 is also 83.6% identical to Japanese flounder HSC71. These results suggest that the amino acid sequence of the cytosolic HSP70 family, HSP70 and HSC71, has been highly conserved among vertebrates. Northern blot analysis showed that HSP70 mRNA was induced with heat shock treatment in the JFE cells in early passage.

Key words: HSP70, heat shock protein, heat-inducible, cDNA, Japanese flounder

Introduction

All organisms synthesize a few evolutionary-conserved proteins called heat shock proteins or HSP, recently called a stress protein, in response to various harmful environmental stresses.^{1–3)} The most abundant and conserved HSP has a molecular mass of about 70 kDa. In eukaryotes, HSP70 are encoded by heat-inducible HSP70 genes and usually their basal expression is negligible. HSP70 is known to act as a molecular chaperon in cells under stress conditions. In fact, *in vitro* studies revealed that the cultured fish cells synthesized HSP70 under adverse conditions such as heat shock, heavy metal ions, and sodium arsenite.¹⁾

The Japanese flounder is one of the most commercially valuable fish and so becomes an important experimental marine fish in Japan. The culture of Japanese flounder has been successful. During the transportation and breeding in the farm, the embryos could be exposed to adverse environmental changes. In the previous report, we cloned the cDNA of HSP70 homolog, a 71 kDa heat shock cognate protein (HSC71), from Japanese flounder embryo and found that the flounder HSC71 mRNA was constitutively

expressed under normal and also stress conditions in the cultured cells in early passage originated in Japanese flounder embryos⁴⁾ in contrast to stress-inducible HSP70 reported on other vertebrates such as human,⁵⁾ bovine,⁶⁾ or rat.⁷⁾ In addition, HSC71 functions as a molecular chaperon.^{1–3)} These findings suggested that the Japanese flounder HSC71 is indispensable for development under normal and also stress conditions. However, in Japanese flounder, little is known about stress-inducible proteins which may have important roles for the accommodation to transitory adverse changes of environment and also for development under stress conditions. Those gene structures in Japanese flounder are also unknown. As a preliminary to resolve the role of stress inducible proteins in the flounder embryos under stress conditions, we decided to clone the stress-inducible HSP70 cDNA, referred to here as Japanese flounder HSP70 cDNA. Nucleotide sequence and expression analyses revealed that the isolated clone represents a heat-inducible HSP70 cDNA.

Materials and Methods

Japanese Flounder Embryos and Culture of cells

Japanese flounder *Paralichthys olivaceus* eggs (embryos

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The following abbreviations are used: HSC, heat shock cognate protein(s); HSP, heat shock protein(s); RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair; kb, kilobase; dNTP, deoxynucleotide triphosphate; JFE cells, cells originated in Japanese flounder embryo; SDS, sodium dodecyl sulphate; SSC, standard sodium chloride/sodium citrate buffer. The DDBJ, GenBank and EMBL accession number for Japanese flounder HSP70 is AB010871.

in eyed period) were provided by Yamagataya Suisan (Nantan, Hyogo, Japan). About 1×10^4 eggs were gently stirred in sea water containing antibiotics (200 units/ml penicillin, 200 $\mu\text{g}/\text{ml}$ streptomycin, and 250 $\mu\text{g}/\text{ml}$ kanamycin), washed in Ca^{2+} and Mg^{2+} -free phosphate-buffered saline (PBS-)containing antibiotics, and mashed in 2 ml of PBS- containing 0.25% trypsin and antibiotics. The suspension was filtered through a stainless-steel mesh. The resulting suspension of cells and small tissue pieces was combined with 10 ml Leibovitz's L15 medium supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, penicillin (100 units/ml), streptomycin (50 $\mu\text{g}/\text{ml}$), and 15% fetal bovine serum (FBS, Biological Industries), and seeded into 25 cm^2 plastic flasks (Corning). The cells, originated in Japanese flounder embryo (JFE cells), were cultured at 20°C in air using the Leibovitz's L15 medium. The primary cultures were maintained for 7–10 days, then subcultured by trypsinization with 0.25% trypsin in PBS- at a split ratio of 1:2 several times until experimental use. A half volume of the medium was renewed every 3–4 days.

Preparation of RNA

Total RNA from JFE cells in early passage was isolated by ultracentrifugation in 5.7 M cesium chloride after homogenization in 4 M guanidinium thiocyanate.⁸⁾ Poly (A)⁺ RNA was enriched by chromatography on oligo (dT) cellulose (Collaborative Biomedical Products) or on Oligotex-dT30 (Takara).

RT-PCR Cloning

To obtain a partial sequence of Japanese flounder HSP70 gene, PCR was conducted with degenerate oligonucleotide primers having the following sequences: 5'-ATCGAYCTSGGSACYACCTACTC-3' as the sense primer and 5'-GCACCGTASSCSACCGCYTCRTC-3' as the antisense primer, where R=A+G, S=G+C, Y=C+T. These correspond to the amino acid sequences, IDLGTYS and DEAVAYGA, respectively, which are identical in the vertebrate HSP70 family such as chinook salmon HSP70,^{*4} human HSP70,⁵⁾ Japanese flounder HSC71⁴⁾ (shown in Fig. 2), bovine HSP70⁶⁾ or HSC71,⁹⁾ and rat HSP70⁷⁾ or HSC71.¹⁰⁾ Single stranded cDNA was synthesized from 1 μg of total RNA of the JFE cells with 200 units of MMLV-RT (Gibco-BRL) in a 20 μl reaction mixture comprising 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2.5 mM MgCl_2 , 0.1 mg/ml 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 for the PCR, which was carried out in a 25 μl solution comprising eight units of Tth DNA polymerase (Toyobo), 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2.5 mM MgCl_2 , 0.1 mg/ml BSA, 0.2 mM each dNTP, and 0.2 μM each primer. The PCR conditions were 30 s at 95°C, 30 s at 56°C, and one min at 72°C for 25 cycles (Astec, Program Temp Control System PC-700). The resultant products of the expected size, approximately 1100 bp, were subcloned in pBluescript II KS⁺ (Stratagene). DNA sequencing analysis revealed that there was a cDNA fragment of HSP70-like gene in the pool of the

PCR products.

cDNA Cloning and Sequencing

The synthesis of double-stranded cDNA was accomplished with a ZAP cDNA Synthesis Kit (Stratagene) using poly (A)⁺ RNA prepared from JFE cells heat-shocked at 37°C for 20 min by chromatography on oligo (dT) cellulose. The cDNAs were size-fractionated on a CHROMA SPIN-100 column (Clontech) and then adapter-ligated prior to the generation of an oligo (dT) primed library in Uni-ZAP/EcoRI/XhoI/CIAP (Stratagene). The cDNA library was screened with a cDNA fragment obtained on PCR as a probe. Plaque hybridization was performed at 42°C in a solution comprising 5 $\times$ SSC (1 $\times$ SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 0.1% SDS, 50% formamide, 100 mg/ml denatured salmon sperm DNA, 0.6% Ficoll 400, 0.6% polyvinylpyrrolidone 100, 0.6% BSA, and ³²P labeled probe prepared with a Megaprime DNA labelling system (Amersham International PLC). After hybridization, the filters were washed four times with 2 $\times$ SSC at room temperature and three times with 2 $\times$ SSC, 0.5% SDS at 68°C prior to autoradiography. Plasmids (pBluescript SK⁻) containing the cDNA insert were obtained by *in vivo* excision from positive single plaques according to the manufacturer's instruction (Stratagene). After subcloning into pBluescript II KS⁺, DNA fragments were sequenced by a PCR procedure employing fluorescent dideoxynucleotides and a model 373A automated sequencer (Applied Biosystems Inc.). The sequences reported in this paper were determined on both strands.

Northern Blot Analysis

The JFE cells in early passage were heat-shocked at 37°C for 0, 20 or 40 min. Immediately after the treatment, the cells were collected. Northern blot analysis was carried out using total RNA (10 μg) prepared from the cells. The cloned cDNA fragment of Japanese flounder HSP70 (1898 to 2112 bp) containing 3'-untranslated regions (1921 to 2112 bp) was used as the probe for hybridization. As an internal control, the cDNA fragment of Japanese flounder HSC71 containing 3'-untranslated regions⁴⁾ was used as the probe for hybridization. Hybridization was performed under the same condition described above.

Results and Discussion

Cloning and Sequence of the Full-length cDNA for HSP70

An RT-PCR strategy for cloning a partial cDNA of Japanese flounder HSP70 successfully yielded a cDNA fragment of approximately 1100 bp. DNA sequencing analysis revealed that the fragment was homologous to heat-inducible mammalian HSP70 gene⁵⁻⁷⁾ (data not shown). In order to obtain a full length cDNA of flounder HSP70, a cDNA library of heat-shocked JFE cells in early passage was screened with the PCR-derived cDNA fragments as a probe. By screening of 10⁵ independent recombinant phages, hundreds of positive clones were isolated. DNA sequencing analyses on both 5' and 3'-end of ten clones

*4 M. T. Hargins, D. D. Dauble, S. Howard, P. Candido, E. Hickey, and L. A. Werber: The DDBJ, GenBank and EMBL accession number for chinook salmon HSP70 is Q91233 (unpublished).

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-86   AGCACAAACAAAGAGCTGAAACAGAGTCTGCTGAGATCCTTCAACATCTCGGAGCTACACATTATCTCTGAAAGAACAAACAGG   -1
  1  M S S A K G I S I G I D L G T T Y S C V G V F Q H G K V E I   30
  1  ATGTCTTCAGCGAAGGGAATATCCATTGGCATTGATTTGGGAACCACTATTCTTGTGTGGGGTTTCCAACATGGCAAAGTGAGATC   90
 31  I A N D Q G N R T T P S Y V A F T D T E R L I G D A A K N Q   60
 91  ATTGCCAACGACGAGGCAACAGGACAACCTCCAGCTATGTGGCCTTTACCGACACAGAGGCTCATTGGAGATGCTGCCAAGAACCAG   180
 61  V A M N P T N T I F D A K R L I G R K F N D S I V Q S D M K   90
181  GTTGCCATGAACCCACCAATACAATATTTGACGCCAAGCGACTAATAGGAAGAAAGTTAATGACTCCATCGTCCAGTCCGACATGAAA   270
 91  L W P F K V I S D N G K P K V Q V E Y K G E T K A F Y P E E   120
271  CTTTGGCCTTTCAAGGTGATCAGTGATAACGGAAAGCCAAAGTTCAAGTGGAGTATAAAGGCGAAACCAAGGCCTTCTACCTCGAAGAA   360
121  I S S M V L V K M K E I A E A Y L G Q K V S N A V I T V P A   150
361  ATCTCGTCAATGGTCTGTGTTAAAATGAAGGAGATAGCTGAGGCTACCTGGGACAAAAGTTTCAAATGCAGTCATCACAGTGCCAGCT   450
151  Y F N D S Q R Q A T K D A G V I S G L N I L R I I N E P T A   180
451  TATTTCAATGATTCTCAGAGGCAAGCCACTAAGGATGCTGGAGTGATCTCTGGTCTGAATATTCTGAGGATCATCAACGAACCCACAGCA   540
181  A A I A Y G L D K G K R G E R N V L I F D L G G G T F D V S   210
541  GCAGCGATTGCTACGGCTTAGATAAAGGTAAGAGGAGAGCGCAACGTGCTCATCTTTGATCTCGGTGGAGGCACCTTTGACGTGTCC   630
211  I L T I E D G I F E V K A T A G D T H L G G E D F D N R M V   240
631  ATCTGACCATTTGAGGACGGCATCTTTGAGGTAAGGCACTGCAGGAGACACACCTTGGTGGGAGGACTTTGACAATCGGATGTGC   720
241  S H F L E E F K R K Y K K D I S Q N K R A V R R L R T A C E   270
721  AGTCACTTCTCGAGGAATTTAAAAGAAAGTACAAGAAAGACATCAGCCAAAATAAGAGAGCAGTTAGGAGACTGCGCAGACGTTGTGAG   810
271  R A K R T L S S S T Q A S I E I D S L F E G I D F Y T S I T   300
811  AGAGCGAAGAGGACCTGTCTCAAGCACCCAGGCGCAGTGAATCAGCTCTCTGTTTGGGGCATCGACTTCTACACCTCCATCACA   900
301  R A R F E E L N S D L F R G T L E P V E K A L Q D S K M D K   330
901  AGGGCACGCTTTGAGGAGCTCAACTCAGATCTCTTCAAGGGAACACTGGAGCGCAGTGGAGAAAGCCCTACAAGATCCAAAGTGGATAAG   990
331  S K I H E I V L V G G S T R I P K I Q K F L Q D F F N G R E   360
991  TCCAAGATCCATGAAATCGTCTGTTGGTGGCTCCACAAGAATCCCAAAATCCAGAAGTTCTTGCAAGATTTCTTAAATGGCAGAGAA   1080
361  L N K S I N P D E A V A Y G A A V Q A A I L M G D T S E N V   390
1081  CTGAACAAGAGTATCAACCCAGATGAAGCTGTGGCTTATGGTGCAGCAGTCCAGGCTGCTATCTCATGGGTGACACTTCGGAGAACGTC   1170
391  Q D L L L L L D V A P L S L G I E T A G G V M T P L I K R N T   420
1171  CAAGATCTGCTGCTGCTGGAGCTGGCTCCCTGCTCTCTGGGTATTGAGACTGCTGGTGGAGTATGACACCTCTGATCAACGCAACACA   1260
421  T I P T K Q T Q I F T T Y S D N Q P G V L I Q V Y E G E R A   450
1261  ACTATCCCACCAAGCAAACCCAGATCTTCAACCACTACTCAGATAACCAAGCTGGTGTGCTAATTGAGTGTATGAGGGCGAGAGAGCC   1350
451  M T K D N N L L G K F E L A G I P P A P R G V P Q V E V T F   480
1351  ATGACCAAGGACAACAACCTCTGGGCAAGTTTGAGCTCGTGGCATCCTCTGCTCCCGAGGCGTACCCAGGTGGAGGTAACCTTTC   1440
481  D I D A N G I L N V S A V D K S T G K E N K I T I T N D K G   510
1441  GACATTGATGCCAACGGAATTTCTGAACGTGTCTGCTGCACAAAAGCACCGGCAAGAAAACAAAATCACCATCACAAATGACAAAGGC   1530
511  R L S K Q E I E Q M V Q D A E K Y K A D D E L K R D T I T A   540
1531  CGCCTCAGCAACAGGAGATTGAGCAGATGGTACAGGATGCTGAGAAGTACAAGGCTGATGACGAGCTGAAGAGGGACACGATTACAGCA   1620
541  R N S L E S Y V Y H M K S S V E D D S M K G K I Q E E D K K   570
1621  AGGAACCTACTGGAGAGTTACGTTTACCACATGAAGAGCAGCGTTGAGGATGACAGTATGAAAGGAAAGATTGAGGAGGAGGACAAAAA   1710
571  I V I D K C N Q T I S W L E N N Q L A E K D E Y E H Q Q K E   600
1711  ATTGTCAATGACAAGTGCAACCAAGCAATCTCTGGCTGGAGAACAACCAAGCTGGCAGAGAAGGACGAGTATGAGCACCAAGCAGAGGAA   1800
601  L E K V C N P I V T K L Y Q G A A P P P G G S S G H A G G N   630
1801  CTAGAGAAGGTGCAATCCGATTGTGACAAAGCTGTACAGGAGCGGCACCACCAGGAGGAGCAGCGCCATGCAGGAGGCAAC   1890
631  A Q G P T I E E V D   640
1891  GCTCAGGTCCTCACTATTGAGGAGGTGGACTAATAACATGAAAGTCATGTCAATATTTACATGGCTATGATGTTTGAACATTAAATTGA   1980
1981  GTTTCTTTCTCTGCTGCATCCACTTTTACTCATTATAATTTATTTCTGCTTTGTTCACTTTTCTTGTGTCCACTCAGATTGTATTTT   2070
2071  AAATAAAATTTTATGAAAAACAAAAAATAAAAAAAAAAAAAAAAAAAAAA   2112

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Fig. 1. Nucleotide and deduced amino acid sequences of the HSP70 cDNA of Japanese flounder. The position of initiation (base pairs 1 to 3), termination (1921 to 1923) codons, and a putative polyadenylation signal (2072 to 2077) in the sequence is underlined.

selected at random revealed that they encoded the same gene (data not shown). As a result, we determined the nucleotide sequences of the longest clone.

The cDNA of Japanese flounder HSP70 consists of 2198 nucleotides with poly (A) tail (Fig. 1). The in-frame stop codon in the 5' and 3' regions of the cDNA indicated that the clone contains the entire coding regions. The coding region includes 1920 nucleotides and corresponds 640 amino acids with a predicted mass of 70.6 kDa.

Figure 2 shows the comparison of the nucleotides sequence of HSP70 from Japanese flounder with those of other vertebrates. Japanese flounder HSP70 contains a EEVD (Glu-Glu-Val-Asp) peptide motif at C-terminal end which is the common feature of the cytosolic HSP70

family.¹⁾ Comparison with protein sequences in the SWISS-PROT database by FASTA indicated that Japanese flounder HSP70 is 86.0%, 83.8%, 83.6%, and 82.8% identical in primary structure to chinook salmon HSP70, bovine HSP70,⁶⁾ human HSP70,⁵⁾ and rat HSP,⁷⁾ respectively. Obviously the HSP70 amino acid sequence has been highly conserved among various vertebrate taxa. On the other hand, similarity to the HSC71s, which are heat-noninducible HSP70 homologs and expressed constitutively, are also high. Similarity to Japanese flounder HSC71 (Fig. 2),⁴⁾ human HSC71,¹¹⁾ bovine HSC71,⁹⁾ and rat HSC71¹⁰⁾ amount to 83.6%, 83.6%, 82.9, and 83.7%, respectively. These results suggest that the amino acid sequence of the cytosolic HSP70 family, HSP70 and

jfHSP70	MSSAKGISIGIDLTGTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVAMNPTNTIF	70
sHSP70	P.....N..V..	70
hHSP70	...AAA.....L..Q..V..	68
jfHSC71	...PAV.....S.....V..	68
jfHSP70	DAKRLIGRKFNDSIVQSDMKLWPFKVISDNQPKPVQVEYKGETKAFYPEEISSMVLVKMKEIAEAYLGQK	140
sHSP70	QV..A...H....V..G.....D...N.S.N.....R.....	140
hHSP70	G.PV.....H...Q..N.GD.....S.....T.....YP	138
jfHSC71	R.D.AV.....H...N..N.STR.....S.S.....T.....KT	138
jfHSP70	VSNAVITVPAYFNDSQRQATKDAGVISGLNLIINEPTAAAIAYGLDKGKRGERNVLIFDLGGGTFDVS	210
sHSP70	A..V.....M...MSR.....	210
hHSP70	.T.....A..V.....RTGK.....	208
jfHSC71	.N...V.....T....V.....KVGS.....	208
jfHSP70	ILTIEDGIFEVKATAGDTHLGGEDFDNRMVSHFLEEFKRKYKDISQNKRAVRRLRTACERAKRTLSST	280
sHSP70	L...V.....H.....L.....S	280
hHSP70	...D.....L.N..V.....H.....	278
jfHSC71	N..IA.....D.....	278
jfHSP70	QASIEIDSLFEGIDFYTSITRARFEELNSDLFRGTLEPVEKALQDSKMDKSKIHEIVLVGGSTRIPKIQK	350
sHSP70	MC.....R.A...AQ..DV.....V..	350
hHSP70	...L.....C....S.....R.A.L..AQ..DL.....V..	348
jfHSC71	Y..V.....A.....D...S.R.A...GQ..D.....	348
jfHSP70	FLQDFENGRELNKSINPDEAVAYGAAVQAAILMGDTSENVQDLLLLDVAPLSLGIETAGGVMTPLIKRNT	420
sHSP70	L.....I....S..K.....A.....	420
hHSP70	L.....D.....K.....L.....A....S	418
jfHSC71	L.....R.....S..K.....T.....V.....	418
jfHSP70	TIPTKQTQIFTTYSDNQPGVLIQVYEGERAMTKDNNLLGKFELAGIPPAPRGVPQVEVTFDIDANGILNV	490
sHSP70	...S...T.....M.....S.....I.....	490
hHSP70	R...S.....I.....	488
jfHSC71	T.....R.....T.....I.....M..	488
jfHSP70	SAVDKSTGKENKITITNDKGRLSKQIEIQMVQDAEKYKADDELKRDITARNLSLEYVYHMKSSVEDDSM	560
sHSP70	ED..R....D....E.DAQ.EK.A.K.....ANF.....N.	560
hHSP70	T.T.....A.....E...R...E.....E.VQ.ERVS.K.A...AFN...A...EGL	558
jfHSC71	..A.....ED..R...E.....TE.DVQ..KVS.K.GV...AFN...T...EKL	558
jfHSP70	KGKIQEEDKKIVIDKCNQTISWLENNQLAEKDEYEHQKQEKVCNPIVTKLYQGAAP-----PPG	621
sHSP70	...SQ...K.V.R.D.....GD.E....L.....Q..I....QGGM-----T.	621
hHSP70	...S.A...K.L...QEV...DA.T....F..KR...Q....ISG....GG-PG-----G.	621
jfHSC71	A...SD...QKIL...EV...DK..T.....I....S.GGMPGGMPEGM.G.	628
jfHSP70	GS----SGHAGGNAQGPTIEEVD	640
sHSP70	CCGDQARTSS.DSS.....I.	644
hHSP70	FGAQG--PK..SGS.....	641
jfHSC71	FGGAGGAAPGA.S-S.....	650

Fig. 2. Protein comparison. Deduced amino acid sequence of a protein encoded by the cloned Japanese flounder cDNA (jfHSP70) is compared to that of chinook salmon HSP70 (sHSP70), human heat-inducible HSP70 (hHSP70),⁵⁾ and Japanese flounder HSC71 expressed constitutively (jfHSC71).⁴⁾ Only different amino acids are indicated and hyphens mark deletions.

HSC71, has been highly conserved among vertebrates.

Heat-inducible Expression of Japanese Flounder HSP70 mRNA

To investigate the expression pattern of Japanese flounder HSP70 mRNA, Northern blot analyses of total RNA from normal or heat-shocked cells were conducted (Fig. 3). The probe for hybridization was prepared from the cloned HSP70 cDNA fragments (1898 to 2112 bp) that consisted of mainly 3' untranslated regions (1921 to 2112 bp, Fig. 1) to tighten the specific affinity on HSP70 mRNA rather than HSC71 mRNA. In those regions, the similarity of Japanese flounder HSP70 to Japanese flounder HSC71

was 56% by multiple alignment using malign. Little signal was observed on RNA isolated from unheat-shocked cells. On the other hand, a significant signal increase of approximate 2.2 kb and 2.4 kb bands were observed on RNA after heat shock for 20 min. After heat shock for 40 min, the signal of 2.2 kb band was decreased and the signal of 2.4 kb band was increased. The possible reason of the size heterogeneity of Japanese flounder HSP70 mRNAs is that the differences in length of 3'-untranslated regions. At present, it is unclear that the exact reason for differences in the expression patterns of 2.2 and 2.4 kb HSP70 bands. As shown in Fig. 3, Japanese flounder HSC71 mRNA was constitutively expressed under normal (0 time) or heat-

Fig. 3. RNA expression analysis. Northern blot analyses were carried out using 10 μ g total RNA isolated from control (0 min) and heat-shocked (37°C for 20 or 40 min) Japanese flounder cells in early passage. The probe for hybridization was prepared from the cloned HSP70 cDNA fragments (1898 to 2112 bp) containing 3'-untranslated regions (1921 to 2112 bp). As an internal control, the cDNA fragment of Japanese flounder HSC71 containing 3'-untranslated regions⁴⁾ was used. Hybridization was done under the same condition described above.

shocked conditions (20 and 40 min).

Although the patterns in expression of HSP70 and HSC71 are different (Fig. 3), it was shown that they have similar structure and function as molecular chaperons in cultured cells of mammals.¹²⁾ In yeast, genetic analysis has shown that HSP70 compensated for those growths at higher temperature on behalf of the cognate genes which expressed constitutively.¹³⁾ These findings and high similarity of HSP70 and HSC71 suggested that synthesized HSP70 and HSC71 under stress conditions may have similar functions in Japanese flounder.

The analyses on expression patterns and functions of Japanese flounder HSP70 and HSC71 in the embryos during development under normal and stress conditions are now in progress.

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