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Structure and Expression of Carp Mitogen-Activated Protein Kinases Homologous to Mammalian JNK/SAPK[1]

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Two distinct stress-activated protein kinase (JNKa and b) cDNAs were isolated from a carp ovary cDNA library. These cDNAs contained a full-length open reading frame encoding 427 amino acid residues with a predicted mass of 48.6 kDa. The deduced amino acid sequences of JNKa and b were 95.8% identical, with 18 residues replaced, and showed a high degree of sequence similarity to mammalian JNK/SAPK subgroup including the common dual phosphorylation motif of TPY. By Northern blot analysis, the carp JNKs were found to be abundant in the brain and ovary. Detailed study by RT-PCR assay revealed ubiquitous expression of JNKb, although expression of JNKa was specific to the brain and ovary. The high level expression of both JNKa and b in the ovary implies that JNKs play an important role in egg maturation or ectogenetic early development.

Key words: carp, cDNA cloning, JNK, MAP kinase superfamily, stress-activated protein kinase.

Cellular responses to extracellular signals, such as mitogens and environmental stresses, require distinct members of the mitogen-activated protein kinase (MAP kinase) superfamily for the transduction of different information from the cell surface to the nucleus (*1-3*). While the ERK subgroup of the MAP kinase family is involved in responses to growth factors (*4, 5*), the JNK and p38 subgroups are key signal-transducers of responses to several stresses and pro-inflammatory cytokines, such as UV irradiation, hyperosmolarity, heat shock, endotoxin, TNF, and IL-1 (*6, 7*).

The cDNAs of JNK, also termed SAPK, have been identified in human and rat (*8-11*). DNA sequence analysis of these cDNAs revealed that JNK shares some similarity with yeast HOG1, which had been identified as a MAP kinase essential for growth in a hyperosmolar environment (*12*). Complementation of HOG1-mutation by introducing JNK1 cDNA supported the functional similarity between JNK1 and HOG1 (*13*). Indeed, JNK1 was activated by hyperosmotic stress in mammalian cells (*13, 14*). However, a significant difference is seen between JNK1 and HOG1 in the dual-phosphorylation motif, TPY and TGY, respectively. JNK or SAPK homolog with the TPY motif has not been identified in lower animals or yeast, while mammalian p38s (*15, 16*) and frog Mpk2 (*17*) share with HOG1 the same motif, TGY. These findings are of interest in relation to the molecular evolution of the JNK/SAPK subgroup of the MAP kinase superfamily.

In order to identify a JNK/SAPK homolog in a lower vertebrate, we attempted to isolate the cDNA and to determine the structure of fish JNK/SAPK. The results should throw light on the evolution of the MAP kinase superfamily. Furthermore, the use of fish as a source of cDNA for cloning is expected to provide a good *in vivo* tool for laboratory research because of its ectogenetic early development in addition to its transparency and fecundity (*18, 19*). These advantages of fish as a vertebrate model should make it possible to investigate the function of JNK/SAPK in an early development, whereas this kind of study using mammals such as the mouse and the rat involves difficulties in direct observation of embryos. Here we report the molecular cloning of two stress-activated protein kinase (JNKa and b) cDNAs from carp. Tissue differences in expression of JNKa and b were also examined.

MATERIALS AND METHODS

Preparation of RNA—Total RNA from either a carp epithelial cell line, EPC (*20*), or various tissues of a female carp (*Cyprinus carpio* L.) was isolated by ultracentrifugation in 5.7 M cesium chloride after homogenization in 4 M guanidinium thiocyanate (*21*). Poly(A)$^+$ RNA was enriched by chromatography either on oligo(dT) cellulose (Collaborative Biomedical Products) or on Oligotex-dT30 (Takara).

RT-PCR—Two pairs of oligonucleotide primers were

[1] The DDBJ, GenBank, and EMBL accession numbers for carp JNKa and JNKb are D83273 and AB001744, respectively.

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Abbreviations: ERK, extracellular signal-regulated kinase; HOG1, high osmolarity glycerol response 1; IL-1, interleukin-1; JNK, c-Jun NH_2-terminal kinase; LPS, lipopolysaccharide; MAP kinase, mitogen-activated protein kinase; ORF, open reading frame; RT-PCR, reverse transcription-polymerase chain reaction; SAPK, stress-activated kinase; TNF, tumor necrosis factor; UTR, untranslated region; UV, ultraviolet.

prepared: J-1 pair (sense: 5′-AANCGATAYCARAAYYT-3′, and antisense: 5′-NAWNACNAGCATYTT-3′) and J-2 pair (sense: 5′-GTNGCNATHAARAARCT-3′, and antisense: 5′-CAAYTGRTCDATAT-3′); (abbreviations according to the IUPAC code). These primers were designed from the well-conserved sequence of the MAP kinase family, human JNK1 (*8*), mouse p38 (*15*), mouse p42 (*22*), and yeast HOG1 (*12*). The J-1 pair corresponds to the amino acid sequences (K, E, N, P)RY(Q, N, T)(N, D)L (residues 24-29 of human JNK1) and KML(V, T)(I, L, F) (residues 300-304). The J-2 pair corresponds to the amino acid sequences VA(I, V)KK(L, I) (residues 52-57 of human JNK1) and (Y, H)(I, V, L)(D, H)Q(L, W, F) (residues 229-233).

Single-stranded cDNA was synthesized from 1 μg of total RNA with 200 units of MMLV-RT (Gibco-BRL) in 20 μl of a reaction mixture containing 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 in the first PCR, which was carried out in a 25 μl solution containing 8 units of ΔTth 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 of the J-1 pair. The second PCR was performed using 1 μl of the first PCR reaction mixture in 50 μl containing 2.5 units of ΔTth DNA polymerase, 25 μM each primer of the J-2 pair, and 0.2 mM each dNTP. The conditions of both PCRs were 30 s at 95°C, 1 min at 56°C, and 2 min at 72°C for 25 cycles (Astec, Program Temp. Control System PC-700).

cDNA Cloning—Synthesis of double-stranded cDNA was accomplished with a ZAP-cDNA Synthesis Kit (Stratagene) using poly(A)$^+$ RNA prepared from the ovary by chromatography on oligo (dT) cellulose. cDNAs were size-fractionated with a CHROMA SPIN-100 column (CLONTECH) and adapter-ligated prior to generation of oligo (dT) primed library in Uni-ZAP/*Eco*RI/*Xho*I/CIAP (Stratagene).

The cDNA library was screened with a 549 bp fragment obtained by PCR as a probe. Plaque hybridization was done at 42°C in a solution containing 5×SSC (1×SSC=150 mM sodium chloride, 15 mM sodium citrate, pH 7.0), 0.1% SDS, 50% formamide, 100 μg/ml denatured salmon sperm DNA, 0.6% Ficoll 400, 0.6% polyvinylpyrrolidone 100, 0.6% BSA, and ^{32}P-labeled probe prepared by use of the Megaprime DNA labeling system (Amersham International plc). After hybridization, the filters were washed four times with 2×SSC at room temperature and three times with 2×SSC, 0.5% SDS at 65°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 (Stratagene), DNA fragments were sequenced by a PCR procedure employing fluorescent dideoxynucleotides and a model 373A automated sequencer (Applied Biosystems). The sequences reported in this paper were determined on both strands.

Southern and Northern Blot Analyses—A genomic Southern experiment was performed by the standard procedure (*21*) using 10 μg of carp genomic DNA digested with restriction enzymes (Toyobo). Northern analysis was carried out using poly(A)$^+$ RNA prepared by Oligotex-dT30 (Takara). In both analyses, the probes for hybridization were prepared from the 549 bp cDNA fragment, or 3′-untranslated region of the two distinct JNK cDNAs. Hybridization was done under the same conditions described above.

Detection of Two Distinct JNK mRNAs—RT-PCR assay was performed with primers having the following sequences: The sense primer (J5′) specific to nucleotides 212-231 of the ORF of both JNK a and b cDNAs is 5′-ACAGAGAGCTGGTGCTCATG-3′. The antisense primers specific to nucleotides 660-679 of the ORF of JNK a and b cDNA are 5′-CAGGAAAAAGGATTTTGTGA-3′ (Ja) and 5′-CTGGAAACAACACACTACCT-3′ (Jb), respectively

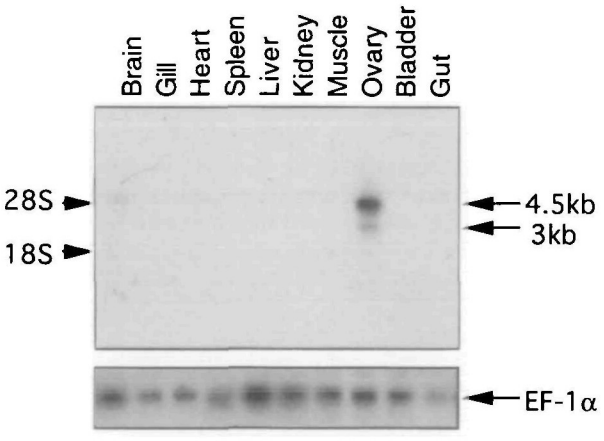

Fig. 1. **Tissue distribution of carp JNK mRNA.** Poly(A)$^+$ RNA samples were prepared from the brain, gill, heart, spleen, liver, kidney, muscle, ovary, gall bladder, and gut. Each RNA sample (2 μg) was separated by 1% agarose-formaldehyde gel electrophoresis, transferred onto a nylon membrane and fixed. The JNK cDNA fragment derived by RT-PCR cloning was used as a probe. After hybridization, the membrane was washed at 65°C in 2×SSC containing 0.5% SDS. The blot was reprobed with medaka elongation factor-1α cDNA as an internal control. The positions of 28S and 18S rRNAs are indicated on the left by arrowheads.

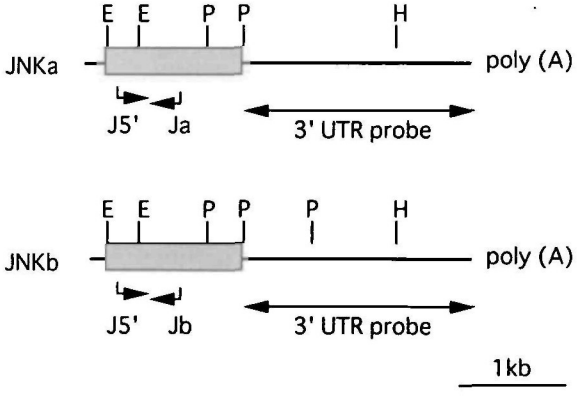

Fig. 2. **Restriction map of the cDNA clones of carp JNKa and b.** Filled boxes indicate the ORFs. Restriction endonuclease cleavage sites are indicated as follows: H, *Hin*dIII; P, *Pst*I; E, *Eco*RI. Arrows below the ORFs show the position of primers, Ja, Jb, and J3′, which were used in RT-PCR assay (Fig. 6). The 3′-untranslated region (UTR) used as a probe for Southern and Northern blot analyses is illustrated by a line with double arrowheads.

(Fig. 2). Fifty nanograms of poly(A)$^+$ RNA purified by Oligotex-dT30 (Takara) was transcribed into cDNA with 5 units of AMV-RT (Takara) in 20 μl of a reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 5 mM $MgCl_2$, 1 mM each dNTP, 2.5 μM random nonamer, and 5 units of ribonuclease inhibitor (Takara). One-tenth of the resultant cDNA was used as a template for PCR, which was carried out in a 10 μl solution containing 0.5 units of recombinant Taq DNA polymerase (Takara), 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, 0.2 μM each primer of J5′ and either Ja or Jb. For an internal control, β-actin mRNA was amplified with a pair of oligonucleotides (sense: 5′-TGCCATCCAGGCTGTGCTG-3′, and antisense: 5′-CCATCTCCTGCTCGAAGT-3′) as primers. The conditions of PCRs were 30 s at 94°C, 30 s at 60°C, and 1 min 30 s at 72°C for 30 cycles (Astec, Program Temp. Control System PC-700). Amplified PCR fragments were stained with ethidium bromide after electrophoresis on a 1% agarose gel.

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JNKa   carp     1 MNKNKREKEF YSVDVGDSTF TVLKRYQNLR PIGSGAQGIV CSAYDHNLER NVAIKKLSRP
JNKb   carp     1 .......... .......... .......... .......... .......... ..........
JNK1   human    1 .SRS..DNN. ...EI..... .........K .......... .A...AI... ..........
JNK2   human    1 .SRS.CDSQ. ...Q.A.... .......Q.K .......... .A.F.TV.GI S..V......
SAPKβ  rat      1 .S.S....Q. ...E..... .........K .......... .A...AV.D. ..........
SAPKγ  rat      1 .SRS..DNN. ...EIA.... .........K .......... .A...AI... ..........
HOG1   yeast    1 .T---TNE.. IRTQIFGTV. EITN..ND.N .V.M..F.L. ...T.TLTSQ P.....IMK.

JNKa   carp    61 FQNQTHAKRA YRELVLMKYV NHKNIICLLN VFTSQKTLDE FQDVYLVMEL MDANLCQVIQ
JNKb   carp    61 .......... ........C. ......G... ...P....E. .......... ..........
JNK1   human   61 .......... ........C. ......G... ...P..S.E. .....I.... ..........
JNK2   human   61 .......... ......L.C. ......S... ...P....E. .......... .........H
SAPKβ  rat     61 .......... ........C. ......S... ...P....E. .......... ..........
SAPKγ  rat     61 .......... ........C. ......G... ...P..S.E. .....I.... ..........
HOG1   yeast   58 .STAVL...T ....K.LKHL R.E.L...QD I.------.SP LE.I.F.T.. QGTD.HRLL.

JNKa   carp   121 ME-LDHERLS YLLYQMLCGI KHLHSAGIIH RDLKPSNIVV KSDCTLKILD FGLARTAATG
JNKb   carp   121 ..-....... .........T .......... .......... .......... ..........
JNK1   human  121 ..-.....M. .......... .......... .......... .......... .......G.S
JNK2   human  121 ..-.....M. .......... .......... .......... .......... .......C.N
SAPKβ  rat    121 ..-.....M. .......SA. .......... .......... .......... .......G.S
SAPKγ  rat    121 ..-.....M. .......... .......... .......... .......... .......G.S
HOG1   yeast  113 TRP.EKQFVQ .F...I.R.L .YV....V.. ........LI NEN.D...C. .....IQDPQ

JNKa   carp   180 LLMTPYVVTR YYRAPEVILG -MGYQANVDI WSVGCILAEM VRHKILFPGR DYIDQWNKVI
JNKb   carp   180 ...ooo.... .......... -........V ......M... ..GSV....S .H.......
JNK1   human  180 FM.ooo.... .......... -...KE...L ......MG.. .C........ ..........
JNK2   human  180 FM.ooo.... .......... -...KE.... ......MG.L .KGCVI.Q.T .H........
SAPKβ  rat    180 FM.ooo.... .......... -...KE.... ......MG.. .......... ..........
SAPKγ  rat    180 FM.ooo.... .......... -...KE...L ......MG.. .CL....... ..........
HOG1   yeast  173 --..G..S.. ......IM.T WQK.DVE... ..A...F... IEG.P....K .HVH.FSIIT

JNKa   carp   239 EQLGTPTQ-- EFMMKLNQSV RTYVENRPRY TGYSFEKLFP DVLFPADSEH NKLKASQARD
JNKb   carp   239 ......S.-- .......... .......... .......... .......... ..........
JNK1   human  239 ......CP-- ...K..QPT. ........K. A......... .......... ..........
JNK2   human  239 ......SA-- ...K..QPT. .N......K. P.IK..E... .WI..SE..R D.I.T.....
SAPKβ  rat    239 ......CP-- ...K..QPT. .N......K. A......... .S........ ..........
SAPKγ  rat    239 ......CP-- ...K..QPT. ........K. A......... .......... ..........
HOG1   yeast  231 DL..S.-PKD VINTICSENT LKF.TSL.HR DPIP.SER.K --TV------ ----EPD.V.

JNKa   carp   297 LLSKMLVIDA SKRISVEEAL QHPYINVWYD PA-EVEAPPP VITDKQLDER EHTVEEWKEL
JNKb   carp   297 .......... ......D... .......... ..-....... L.I....... ..........
JNK1   human  297 .......... ......D... .......... .S-.A..... K.P....... ...I......
JNK2   human  297 .........P D.....D... R....T.... ..-.A..... Q.Y.A..E.. ..AI......
SAPKβ  rat    297 .........P A.....DD.. .......... ..-....... Q.Y....... ...I......
SAPKγ  rat    297 .......... ......D... .......... .S-.A..... K.P....... ...I......
HOG1   yeast  278 ..E....F.P K...TAAD.. A...SAPYH. .TD.P--VAD AKF.WHFNDA DLPVDT.RVM

JNKa   carp   356 IYKEVLDWEE RMKNGAIRGQ PSPLGAAVIN GSPQPSSSSS INDVSSMSTE PTVASDTDSS
JNKb   carp   356 .F........ .....V.... ...I...... .......... .......... ..........
JNK1   human  356 .....M.L.. .T...V.... ....-.Q.QQ *
JNK2   human  356 .....M.... .S...VVKD. ..--D...-S SNAT..Q... ...I...... Q.L.......
SAPKβ  rat    356 .....MNS.. KT...VVK.. ...S....-. S.ESLPP... V..I.....D Q.L.......
SAPKγ  rat    356 .....M.L.. .T...V.... .......... ..QH.V..P. V..M.....D ..L...*
HOG1   yeast  336 M.S.I..FHK IGGSDGQIDI SATFDDQ.AA ATAAAAQAQA QAQAQVQLNM AAHSHNGAGT

JNKa   carp   416 LEASAGPLSC CR*
JNKb   carp   416 .......... ..*
JNK2   human  413 .D..T...EG ..*
SAPKβ  rat    415 ........G. ..*
HOG1   yeast  396 TGNDHSDIAG GNKGQRSCSC K*
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Fig. 3. **Comparison of amino acid sequences of carp JNKa and b with those of human, rat, and yeast MAP kinases.** The sequences comprise our results, and those reported for human JNK1 (*8*) and JNK2 (*9, 10*), rat SAPKβ and γ (*11*), and yeast HOG1 (*12*). Identical residues are denoted by periods, and gaps introduced into the sequences are depicted by hyphens. The position of the conserved TPY motif is indicated by shadowing.

RESULTS AND DISCUSSION

The cDNA amplification by RT-PCR using a primer pair (J-1 set) in the first PCR, followed by the second PCR employing the other primer pair, J-2 set, yielded an expected 549 bp band corresponding in size to a potential JNK. Indeed, a homology search with the GenBank database revealed that the cDNA fragment was homologous to the inner part of human JNK1 cDNA (*8*).

We first examined the tissue distribution of JNK mRNA in carp by Northern blot analysis with the PCR fragment as a probe. As a result, expression of 4.5 kb mRNA was detected in the brain and ovary (Fig. 1). The ovary showed a stronger signal of the 4.5 kb transcript than the brain, and in addition a faint signal of a smaller transcript whose length was approximately 3 kb. Therefore, in order to isolate the full length cDNA for carp JNK, we constructed a cDNA library using ovary RNA. The expression of JNK mRNA was undetectable in the gill, heart, spleen, liver, kidney, muscle, gut, and gall bladder by Northern blot analysis.

The cDNA library was screened with the PCR fragment as a probe. Screening of approximately 3×10^5 plaques yielded 11 positive clones. Restriction mapping and sequence analysis of both the 5′- and 3′-ends of the clones showed the presence of two distinct classes, carp JNKa and JNKb (Fig. 2). The amino acid sequence predicted by the nucleotide sequence of the largest clone from one class (carp JNKa) contained 427 residues with a predicted mass of 48.6 kDa (DDBJ accession number D83273). The in-frame stop codons in the 5′ and 3′ regions of the cDNA indicated that this clone contains the entire carp JNK coding region. JNKb cDNA also encoded the same length of ORF, while the nucleotide sequence was different from that of JNKa: The homology in nucleotide sequence of the ORF is 93.6% (DDBJ accession number AB001744).

Multiple alignments of amino acid sequences of carp JNKs (JNKa and b) with those of other animals are shown in Fig. 3. When the sequence of carp JNKa is compared with that of carp JNKb, 95.8% of the residues are identical; among 427 residues in the deduced sequence, only 18 residues are different. MAP kinases are known to be activated through dual phosphorylation of adjacent threonine (Thr) and tyrosine (Tyr) residues (*1*). These putative phosphorylation sites are Thr183 and Tyr185 in both of the carp JNKs. These sites of carp JNKs possess the Thr-Pro-Tyr tripeptide motif, which is conserved in the JNK subgroup of the MAP kinase family (*5-7*). Comparison with protein sequences in the SWISS-PROT database by FASTA indicated that carp JNKa is 87.4, 77.3, 76.8, 83.6, and 86.1%, and carp JNKb is 87.1, 79.4, 78.2, 83.1, and 86.1%, identical in primary structure to human JNK1 (*8*) and JNK2 (*9, 10*), and rat SAPKα, β, and γ (*11*), respectively. The carboxy-terminal region of carp JNKs is 43 and 16 residues longer than those of human JNK1 and rat SAPK γ, respectively, although the homologies are relatively high. When compared in terms of the sequences and lengths at the carboxy-terminal region, carp JNKs are similar to human JNK2 and rat SAPKβ (Fig. 3). These similarities suggest that carp JNKa and b belong to the JNK/SAPK subgroup of the MAP kinase superfamily. It should be noted that carp JNK isoforms have the same length of deduced amino acid sequence, although mammalian JNK/SAPK isoforms differ in length as shown in Fig. 3.

The cDNA fragment isolated by RT-PCR possibly probed both JNKa and b mRNAs in the Northern blot analysis of various tissues (Fig. 1), because of the sequence similarity in the inner part of the ORFs of the cloned JNKa and b cDNA. Actually, multiple bands were observed by Southern blot analysis of carp genomic DNA using the fragment as a probe, indicating that the probe hybridized to both JNKa and b genes (data not shown). In order to clarify whether there was a difference in length between JNKa and b mRNAs, by using the 3′-untranslated regions of JNKa and JNKb cDNAs (Fig. 2) as a probe, we carried out Northern blot analysis with poly (A) rich RNA of the ovary, where two lengths of the transcript, 3 or 4.5 kb, were detected (Fig. 1). The specificity of these probes was confirmed by a genomic Southern blot analysis to distinguish JNKa and b mRNAs (Fig. 4). Figure 5 shows that both of JNKa and b probes strongly recognized the 4.5 kb mRNA, suggesting that the transcripts of JNKa and JNKb do not vary in length. The faint signal of the smaller mRNA of 3 kb (Fig. 1) possibly implies the existence of a related gene, unidentified at this time.

In order to investigate in detail the tissue distribution of JNKa and b mRNAs, we carried out RT-PCR assay, in which a higher sensitivity and specificity of detection could be expected. Two different antisense primers in combination with a common sense primer were employed to distinguish JNKa and b mRNAs. Figure 6 shows that mRNA expression of JNKa and b exhibited different patterns of tissue distribution. JNKb mRNA was expressed in every tissue examined, being most abundant in the ovary. In contrast, the expression of JNKa mRNA was detected only in the brain and ovary. JNKa mRNA was

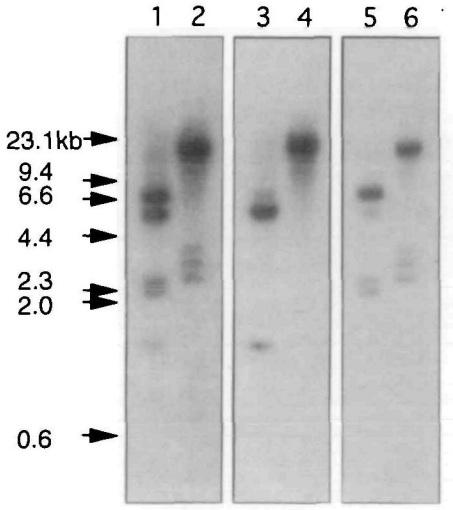

Fig. 4. **Southern blot analysis of carp genomic DNA.** Carp genomic DNA was digested with *Hin*dIII (lanes 1, 3, and 5) and *Eco*RI (lanes 2, 4, and 6), separated by electrophoresis on a 0.8% agarose gel, and transferred onto a nylon membrane. The blots on lanes 3-4 and lanes 5-6 were then hybridized to ^{32}P-labeled JNKa and b fragments of the 3′-untranslated region, respectively (see Fig. 2). The membranes were washed at 68°C in 2×SSC containing 0.5% SDS. A mixed probe of JNKa and b fragments was used for lanes 1 and 2. The positions of DNA size markers are indicated.

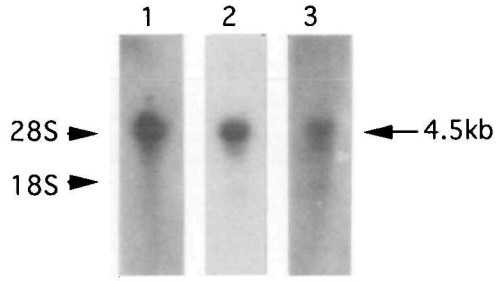

Fig. 5. **Analysis of carp JNKa and b mRNAs in the ovary.** Poly(A)$^+$ RNA from the ovary (1 μg) was separated by 1% agarose-formaldehyde gel electrophoresis, transferred onto a nylon membrane and fixed. The blots on lane 2 and lane 3 were then hybridized to ^{32}P-labeled JNKa and b fragments of the 3′-untranslated region, respectively (see Fig. 2). The membranes were washed at 68°C in 2× SSC containing 0.5% SDS. A mixed probe of JNKa and b fragments was used for lane 1. The molecular size markers consist of carp ribosomal RNAs, indicated by arrowheads.

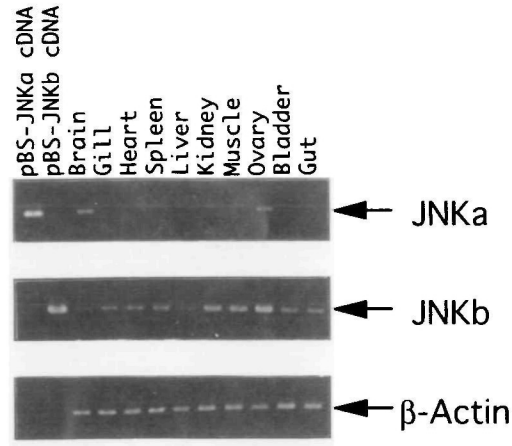

Fig. 6. **RT-PCR assay for detecting JNKa and b mRNAs in various tissues.** The RNA samples prepared from the brain, gill, heart, spleen, liver, kidney, muscle, ovary, gall bladder, and gut were subjected to RT-PCR assays. As an internal control, the expression of β-actin is shown at the bottom. The full-length cDNAs of JNKa and b in pBluescript II were used as templates in PCR as a positive or negative control. All the RNA samples showed no signal when subjected to the reaction without reverse transcriptase. Similar results were obtained in three other experiments.

more abundant in the brain than JNKb mRNA. These findings suggest that carp JNKa and b are expressed under different transcriptional regulations among carp tissues.

In mammals, osmotic stress, heat shock, UV irradiation, IL-1, TNF, LPS, and other stress factors were reported as candidate initiators of stress-responsive signal transduction through JNK/SAPK (*6-11, 13, 14*). Since carp JNKs are highly conserved in structure compared with other animals, they may transduce environmental stress signals to the nucleus. Indeed, they were activated by UV irradiation and hyperosmotic stress (unpublished data). On the other hand, the existence of large amounts of JNKa and b mRNAs in the ovary suggests a role of JNK mRNA or protein in embryos as a maternal factor. Our findings in this study raise the possibility that a female carp equips eggs in the ovary with JNKs to aid their survival when exposed to various environmental stresses during ectogenetic early development. Since the fish embryos do not possess highly-organized endocrinological systems, such as a neurohormonal regulation, cellular responses to environmental stresses seem to be highly dependent on the JNK/SAPK stress-activated kinase pathway. In studies on the function of JNK at an early developmental stage, fish should be a good model for *in vivo* studies because external fertilization and ectogenetic early development, in addition to the transparency, make it possible to observe directly the inside of the embryos (*18, 19*). This would be a great advantage in the application of fish as a vertebrate model, whereas there are difficulties in investigating the physiological functions of JNK/SAPK in mammalian embryos.

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