GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Identification and analysis of the *jnk1* gene in polyploid hybrids of red crucian carp (*Carassius auratus* red var.) and common carp (*Cyprinus carpio* L.)

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Genet. Mol. Res. 13 (1): 906-919 (2014)
Received November 30, 2012
Accepted June 12, 2013
Published February 19, 2014
DOI http://dx.doi.org/10.4238/2014.February.19.1

ABSTRACT. c-Jun N-terminal kinase (JNK) is an important member of the mitogen-activated protein kinase superfamily. The allotetraploid crucian carp is a product of distant hybridization of female red crucian carp with male common carp. It is the first natural case of an allotetraploid with stable genetic characters, including fertility of both female and male animals. In this study, 2 *jnk1* cDNAs (including *jnk1a* and *jnk1b*) have been cloned from the polyploid crucian carp system, consisting of the allotetraploid crucian carp, the triploid crucian carp, and their original parents (red crucian and common carp). We show that *jnk1a* and *jnk1b* represent 2 splice forms arising from the *jnk1* gene. On the basis of the genetic structure of *jnk1a* gene in the polyploid crucian carp system, we demonstrated that the allotetraploid crucian carp is phylogenetically closer to its paternal parent (common carp) than to its maternal parent. We further show a similarity between the triploid crucian carp and its original

female parent (red crucian carp). Comparisons of genetic structures indicated that the *jnk1b* genes of allotetraploid and triploid crucian carp are more similar to those of the original paternal parent rather than the original female parent (red crucian carp). RT-PCR analysis indicated that both the *jnk1a* and *jnk1b* genes are widely expressed in fish embryos and in the adult organs, displaying distinct features of embryonic-stage and organ specificity in the polyploid crucian carp system.

Key words: *jnk1* gene; Polyploidy; Crucian carp; Molecular cloning; Gene expression; Distant hybridization

INTRODUCTION

The c-Jun N-terminal kinase (JNK) is a member of serine/threonine (Ser/Thr) protein kinases known as the mitogen-activated protein kinase (MAPK) family (Davis, 2000; Kyriakis and Avruch, 2001; Weston and Davis, 2002). In mammalians, JNK-mediated c-Jun induction is necessary for proper cell cycle reentry in UV-irradiated fibroblasts (Davis, 2000; Shaulian et al., 2000; Tournier et al., 2000). By contrast, prolonged JNK activation and c-Jun overexpression can result in apoptosis (Shaulian and Karin, 2002). Our studies suggest that in fish, JNK expression is closely associated with embryonic development and organogenesis, and it plays an important role in ovarian differentiation and development (Xiao et al., 2010).

The diploid crucian carp F1 is a product of the distant hybridization of red crucian carp (*Carassius auratus* red var., 2n = 100, ♀) with common carp (*Cyprinus carpio* L., 2n = 100, ♂). As an offspring of F_1, the diploid crucian carp F_2 has the characteristics of forming diploid gametes. As a result allotetraploid crucian carps (4n = 200) are produced and both allotetraploid females and males are able to breed (Liu et al., 2001a,b). The hybridization of diploid crucian carps to form tetraploid crucian carp takes place naturally without requiring artificial induction treatments. To date, the allotetraploid crucian carp has been stably bred for 19 generations from F_3 to F_{21}. It has been suggested that these allotetraploid crucian carps comprising fertile females and males appear to be the first natural case of an allotetraploid vertebrate animal with stable genetic characters (Li et al., 2002; Sun et al., 2003; Liu, 2010). Therefore, allotetraploid crucian carp is regarded as an indispensable animal model for investigating the origin and the evolution of fish polyploidization in nature. With the allotetraploid crucian carp as a male parent and the red crucian carp as a female parent, triploid hybrids (triploid crucian carp, 3n = 150) have been produced in our lab (Shen et al., 2006; Chen et al., 2009). It is believed that because of their fast growth, improved disease resistance, and culinary value, the triploid crucian carps outperform other kinds of fish (Chen et al., 2009). Therefore, the triploid crucian carp has become a popular breeding fish in China.

Distant hybridizations are involved in species formation and facilitate radiating evolution, and it is suggested that such hybridization causes heterologous polyploidy, a potential source of the formation of new species (Mallet, 2007). It has also been noted that via distant hybridization, the genome of one species may be transferred into another one, resulting in phenotypic and genotypic changes in the resultant progenies.

However, little is known about the changes in genetic structure due to distant hybridization in polyploid hybrids. In this study, we cloned the cDNA of the *jnk1* gene in the polyploid

crucian carp system, represented by the allotetraploid crucian carp, the triploid crucian carp, and their original parents, red crucian and common carps. Two subfamilies of the *jnk1* gene, named *jnk1a* and *jnk1b* genes, were obtained, and the sequences of *jnk1a* and *jnk1b* were determined at the cDNA and amino acid levels. Finally, we also studied the expression patterns of the *jnk1a* and *jnk1b* genes during embryonic and adult development of polyploid hybrids.

MATERIAL AND METHODS

Animal materials

The allotetraploid crucian, triploid crucian, red crucian, and common carps were obtained from the Engineering Research Center of Polyploid Fish Breeding in Hunan Normal University, Ministry of Education, China.

Molecular cloning of the full-length *jnk1a* gene

Total RNA was extracted from gonads of the polyploid crucian carp system following the protocol of Xiao et al. (2010). A reverse transcription-linked polymerase chain reaction (RT-PCR) cloning technique was used to clone the full-length *jnk1* cDNA from the allotetraploid crucian carp, the triploid crucian carp, and their original parents (red crucian and common carps). Two jnk1a-P1 oligo primers (see Table 1) were designed to amplify a conserved fragment of 719 bp of the cDNA near the 3'-end of the *jnk1*-coding region by using the goldfish (*C. auratus*) *jnk1* gene (GenBank accession No. EU374209). RT-PCR was conducted with a kit from Invitrogen with 2 µg total RNA; 2 µL RT reaction mixture was mixed with 1 µL 10 µM jnk1a-P1 primers, 10 µL 2X PCR mix, and 7 µL ddH_2O. The PCR had a total volume of 20 µL and was run under the following conditions: 1 hold at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s. The amplified products were gel-purified and cloned into PMD18-T vector (TaKaRa) for DNA sequencing.

Table 1. Oligo primers used for *jnk1* cDNA cloning or RT-PCR analysis.

Name of oligo primers		Sequences
jnk1a-P1	Forward	5'-AAACTTAGCCGACCCTTCC-3'
	Reverse	5'-CGTGCTTGACTCGCCTTC-3'
jnk1a-P2	Forward	5'-AACACCTACACGCTGCTGGCATCATAC-3'
	Reverse	5'-TCTGAAGCCCAGTAACATAGTAGTG-3'
jnk1a-P3	Forward	5'-TGTATGATGCCAGCAGCGTGTAGGTG-3'
	Reverse	5'-TGAGGTGTGAACACATTCAGTAAGCC-3'
jnk1b-P1	Forward	5'-AGAAACTCAGCCGACCTTT-3'
	Reverse	5'-TACAGCCAACAGACCATACAT-3'
jnk1b-P2	Forward	5'-CTGATGGATGCCAACCTCTGC-3'
	Reverse	5'-TGACACCGTTTCTCATCCGTTC-3'
jnk1b-P3	Forward	5'-GGGACCTTCTGTCTAAAATG-3'
	Reverse	5'-CAAATCCCAAGTGACCAAGA-3'
jnk1b-P4	Forward	5'-GTGATTTTGAAAGGTGATCT-3'
	Reverse	5'-CCAGGCAAGGGTGTAGGG-3'
jnk1b-P5	Forward	5'-TGCTTTAGAATGTGAAGGTGAGAGA-3'
	Reverse	5'-AGTGCTCTTAACAGTGGCAATGATAT-3'
jnk1b-P6	Forward	5'-CCCGCCGAGTGGAGGTGTTTTA-3'
	Reverse	5'-GGAGGTGTTTTATTCCGCACAGCATC-3'
β-actin-P	Forward	5'-CCGTGACCTGACTGACTACCTC-3'
	Reverse	5'-ATACCGCAAGATTCCATACCC-3'

The 3'-RACE was performed using Clontech Universal Primer A Mix (UPM) solution (longer primer: 0.4 μM 5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAAC GCAGAGT-3'; shorter primer: 2 μM 5'-CTAATACGACTCACTATAGGGC-3') and primer jnk1a-P2 (see Table 1). The PCR was performed in a 20-μL reaction mix containing 1 μL 3'-cDNA library (Smart Race cDNA Reverse Transcriptase Kit, Clontech Inc.), 1 μL mixed primers UPM and jnk1a-P2, and 10 μL 2X PCR mix. Amplification conditions were: 1 hold at 94°C for 5 min, followed by 5 cycles of 94°C for 30 s and 72°C for 2 min; 5 cycles of 94°C for 30 s, 68°C for 30 s, and 72°C for 2 min; and 25 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 2 min. The PCR products were re-amplified using 1 μL 10 μM primer jnk1a-P2 (Table 1) and Nested Universal Primer A (5'-AAGCAGTGGTATCAACG CAGAGT-3'). The 5'-RACE was performed using the 5-RACE kit. The PCR procedures were the same as described above, using jnk1a-P3 primers (Table 1) and UPM mix solution. Amplified products were gel purified and cloned into PMD18-T vector (TaKaRa) for sequencing.

RT-PCR

Total RNA was extracted from embryos and tissues of the polyploid crucian carp system, comprising the allotetraploid crucian carp, the triploid crucian carp, and their original parents (red crucian and common carps). The embryos were at different stages (2-cell, multiple-cell, blastula, gastrula, neurula, optical vesicle, brain differentiation, muscle differentiation, heartbeat, eye pigmentation, body pigmentation, and hatching). The extracted tissues included heart, muscle, kidney, liver, ovary, and testis.

About 2 μg total RNA was used in a 20-μL reaction volume, and 2 μL reverse-transcription reaction was used in PCRs. In the PCR amplification, β-actin-P primers were used as controls to determine expression of the *jnk1* gene. Both *jnk1*-specific and β-actin-P primers were added into the same PCR mixture, and the reaction was performed for 30 cycles. The resultant PCR products were separated by agarose gel (1.5%) electrophoresis and DNA bands visualized and photographed under UV illumination. More than 3 batch materials were chosen for each test group, and for each batch material, experiments were repeated more than 3 times.

RESULTS

Molecular cloning and sequence analysis of the *jnk1a* gene from the polyploid crucian carp system

Using gene-specific primers (see Table 1: jnk1a-P1, 2 and 3), the full-length *jnk1a* cDNA was cloned from allotetraploid crucian carp (GenBank accession No. JN542469), triploid crucian carp (JN542468), red crucian carp (JN542467), and common carp (JN257262). The *jnk1a* cDNA gene from allotetraploid crucian carp was 1884 bp long containing an open reading frame (ORF) of 1155 bp, coding for 384 amino acids. The full-length cDNA of the *jnk1a* gene was 1425 bp long, containing an ORF of 1140 bp coding for 380 amino acids in triploid crucian carp, 1435 bp containing an ORF of 1140 bp coding for 380 amino acids in red crucian carp, and 1777 bp containing an ORF of 1155 bp coding for 384 amino acids in common carp (Table 2).

Table 2. Full-length cDNAs of jnk1a from allotetraploid crucian carp (AT), triploid crucian carp (3n), red crucian carp (RC), and common carp (CC).

Sample	Full-length cDNAs (bp)	Coding sequence (CDS)	5'-UTM	3'-UTM	Total of amino acids
AT	1884	226-1380	0-225	1381-1884	384
3n	1425	213-1355	0-212	1356-1425	380
RC	1435	224-1366	0-223	1367-1435	380
CC	1777	181-1335	0-180	1336-1777	384

We next compared the different *jnk1a* cDNA sequences from the polyploid crucian carp system (allotetraploid crucian carp, triploid crucian carp, and their original parents) and observed a high level of sequence similarity among them (Figure 1). The JNK1a protein sequences from allotetraploid crucian and red crucian carp were 96.8% identical, and 98.1% identity was observed between the JNK1a proteins from allotetraploid crucian and common carp, and from triploid crucian and common carp. The JNK1a proteins from triploid crucian carp and red crucian were about 100% identical.

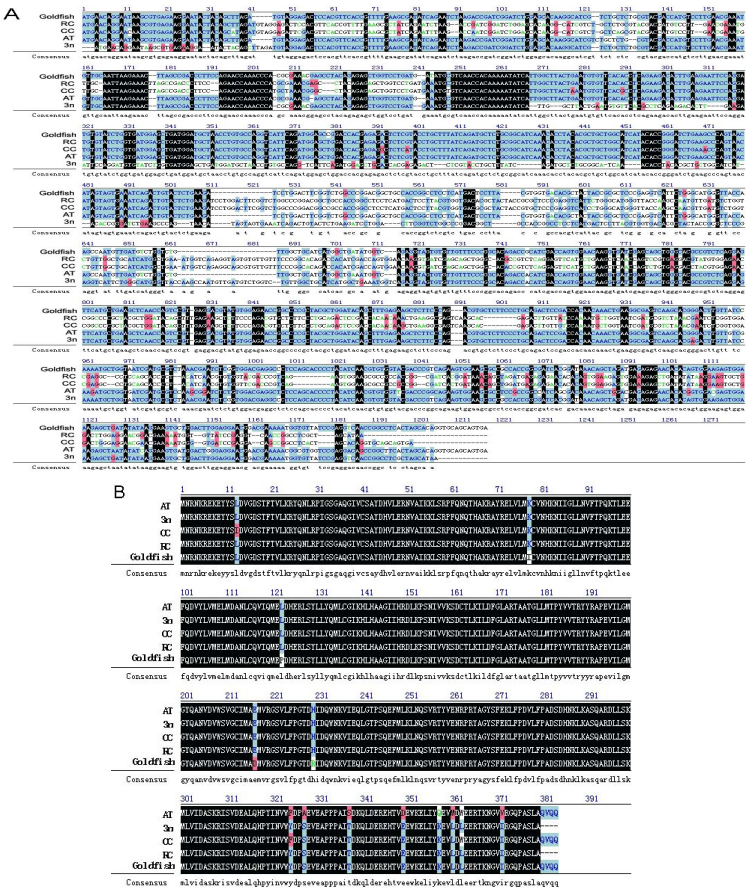

Figure 1. Multiple nucleotide sequence (**A**) and amino acid sequence alignment (**B**). Comparison of the amino acid sequences from JNK1 of gold fish and JNK1a of the polyploid crucian carp system [allotetraploid crucian carp (AT), triploid crucian carp (3n), common carp (CC), and red crucian carp (RC)].

Expression of the *jnk1a* gene in different tissues and embryos of polyploid crucian carps

To test whether the *jnk1a* gene may be involved in the development of polyploid crucian carps, the expression of *jnk1a* was analyzed by RT-PCR, by using the primer jnk1a-P1 (see Table 1). As shown in Figure 2, tissues from liver, testis, ovary, brain, kidney, muscle, heart, gill, and eye had high expression levels of the *jnk1a* gene, whereas its expression was very low in the tail fin of the polyploid crucian carps. In allotetraploid crucian carps, the *jnk1a* mRNA levels in the tissues of liver, ovary, kidney, and heart were similar to the *jnk1a* mRNA levels in the same tissues from their original parents (i.e., red crucian carps and common carps). Expression of *jnk1a* in the brain, gill, and eye was lower in allotetraploid crucian carp than in the other 3 crucian carp species. We also noted that in some tissues, such as testis, ovary, brain, heart, and eye, expression of *jnk1a* was higher in the triploid crucian carp than in red crucian, common, and allotetraploid crucian carps. Both triploid crucian carp and red crucian carp displayed *jnk1a* expression in the muscle that was higher than *jnk1a* expression in the muscles of common and allotetraploid crucian carp.

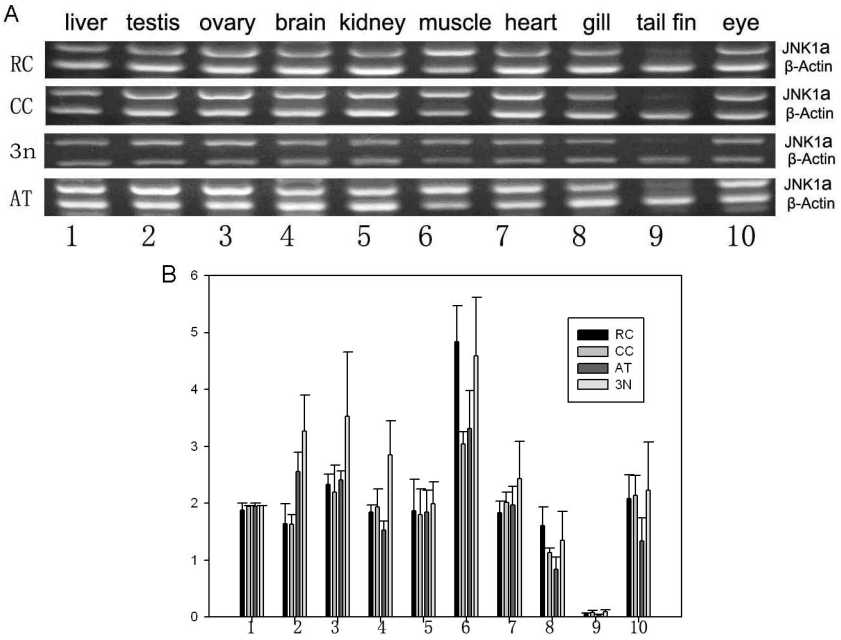

Figure 2. RT-PCR analysis showing the level of the *jnk1a* mRNA in the 10 tissues of the adult fish in the allotetraploid crucian carp (AT), triploid crucian carp (3n), red crucian carp (RC), and common carp (CC) (**A**). Relative level of expression (fold) calculated by dividing the total pixel from each jnk1a mRNA band with the total pixel from the corresponding β-actin mRNA band (**B**). Quantitative results are from three independent experiments.

During embryonic development of polyploid crucian carps, *jnk1a* expression peaked at the multiple-cell stage, and at the blastula and gastrula stages. In the embryos at the stages of blastula, gastrula, neurula, optical vesicle, eye pigmentation, and hatching, expression of *jnk1a* in triploid crucian carp was distinctly lower than expression in the other 3 crucian carp species (Figure 3).

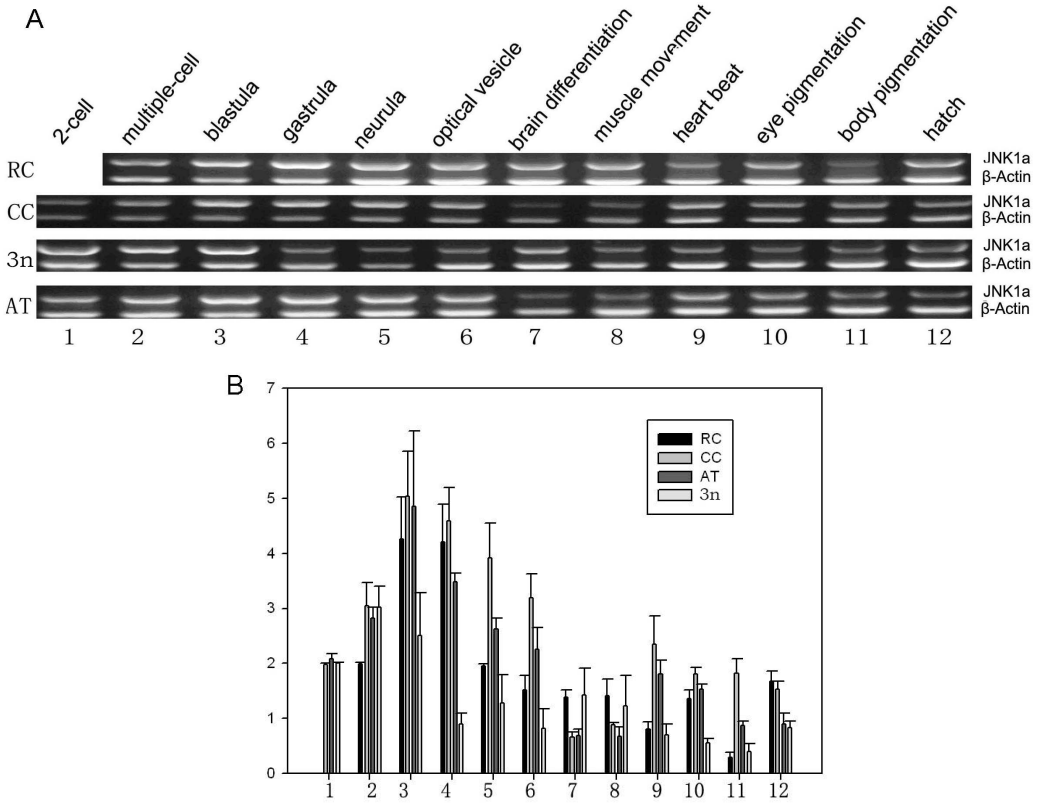

Figure 3. RT-PCR analysis showing the level of the *jnk1a* mRNA in the embryos of allotetraploid crucian carp (AT) and other three fish in the polyploid crucian carp system [triploid crucian carp (3n), common carp (CC) and red crucian carp (RC)] from the stage of cleavage to that of hatching (**A**). Relative level of expression (fold) calculated by dividing the total pixel from each jnk1a mRNA band with the total pixel from the corresponding β-actin mRNA band (**B**). Quantitative results are from three independent experiments.

Molecular cloning of the full-length *jnk1b* cDNA

During the cloning of the *jnk1a* gene from crucian carps, an additional cDNA sequence was obtained and exactly matched the *jnka* gene from Japanese carp (Cyprinus carpio, GenBank accession No. is D83273). Therefore, we used the *jnka* gene sequence of Japanese carp to design 4 primer pairs (jnk1b-P1, 2, 3, and 4) to amplify the cDNA near the 3'-end of the *jnka* coding region (see Table 1). The gene-cloning strategy was the same as that for cloning of the *jnk1a* gene described above. As a result, another gene, named *jnk1b*, was obtained from allotetraploid crucian, triploid crucian, red crucian, and common carp.

The full-length cDNA of the *jnk1b* gene had a length of 3601 bp in allotetraploid crucian carp (GenBank accession No. JN542472), 3761 bp in triploid crucian carp (JN542471), 3619 bp in red crucian carp (JN257261), and 3615 bp in common carp (JN542470). All of the *jnk1b* cDNAs from the polyploid crucian carp system contained an ORF of 1284 bp, coding for 427 amino acids (Table 3).

Table 3. Full-length cDNAs of the *jnk1b* gene from the allotetraploid crucian (AT), triploid crucian (3n), red crucian (RC), and common carp (CC).

Sample	Full-length cDNA (bp)	Coding sequence (CDS)	5'-UTM	3'-UTM	Total of amino acids
AT	3601	215-1498	0-214	1499-3601	427
3n	3761	271-1554	0-270	1555-3671	427
RC	3615	248-1531	0-247	1532-3615	427
CC	3619	237-1520	0-236	1521-3619	427

JNK1b sequences identities were 98.1% between allotetraploid crucian and red crucian carp, 99.0% between allotetraploid crucian and common carp, 99% between triploid crucian and red crucian carp, and 99.5% between triploid crucian and common carp (see Figure 4).

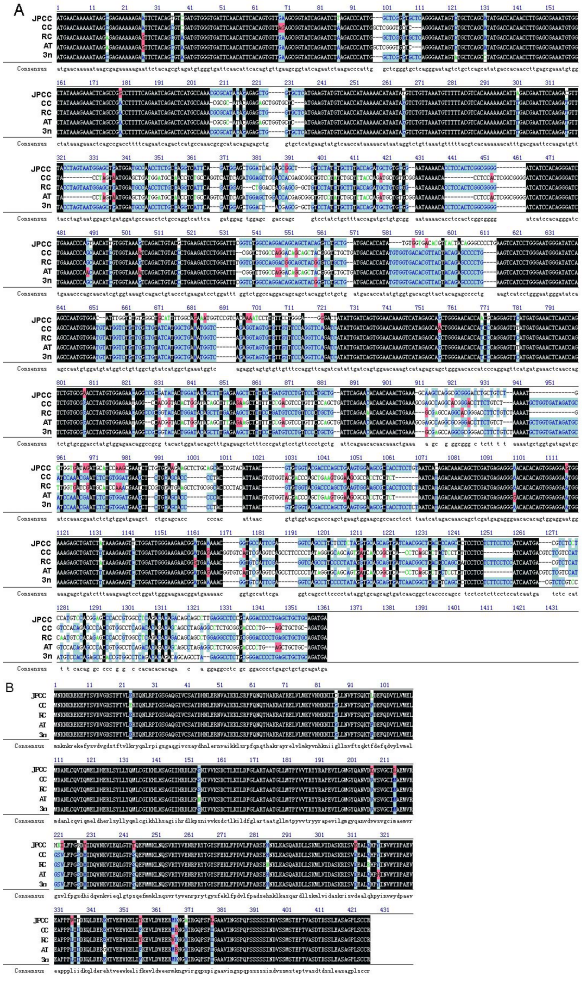

Figure 4. Multiple nucleotide sequence (**A**) and amino acid sequence alignment (**B**). Comparison among the JNKa of Japanese carp (JPCC), the JNK1b of allotetraploid crucian carp (AT) and the other three polyploid crucian carps [triploid crucian (3n), red crucian (RC) and common (CC)].

Expression of the *jnk1b* gene in the tissues and embryos of polyploid crucian carps

jnk1b expression was determined in different tissues and embryos of polyploid crucian carps by RT-PCR with primer jnk1b-P1 (Table 10). This analysis indicated high levels of *jnk1b* expression in the tissues of ovary, brain, and eye for all 4 crucian carp species. In contrast, *jnk1b* mRNA levels were very low in the tissues of liver, kidney, heart, gill, and tail fin for all 4 species. In addition, the *jnk1b* gene was more highly expressed in the ovary than in the testis. For both allotetraploid crucian and triploid crucian carp, expression of *jnk1b* in muscle was lower than that in their original parents (see Figure 5).

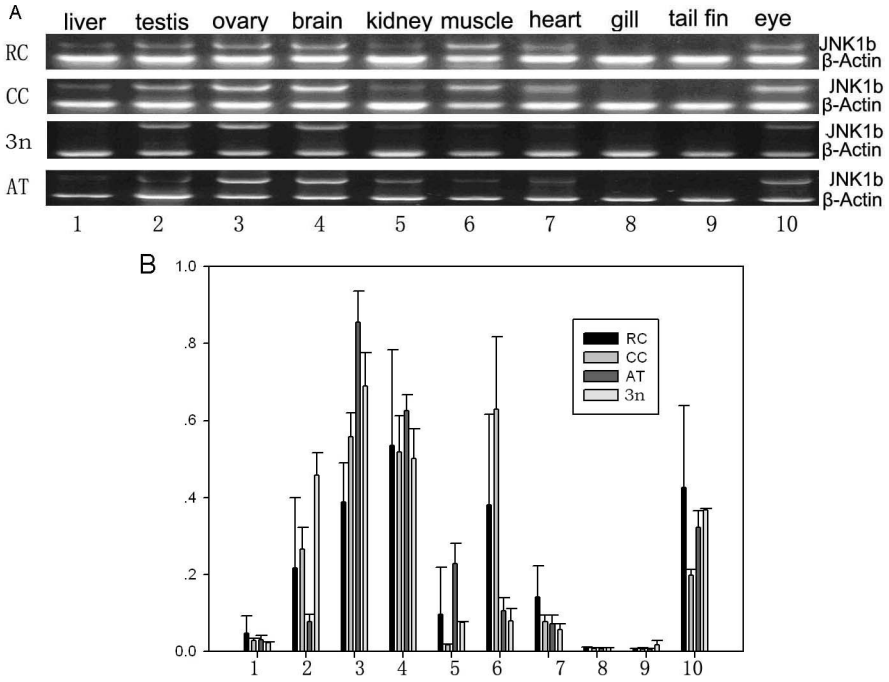

Figure 5. RT-PCR analysis shows that the level of *jnk1b* mRNA in the 10 tissues of the adult fish in the allotetraploid crucian carp (AT), triploid crucian carp (3n), common carp (CC) and red crucian carp (RC) (**A**). Relative level of expression (fold) was calculated by dividing the total pixel from each jnk1b mRNA band with the total pixel from the corresponding β-actin mRNA band (**B**). Quantitative results are from three independent experiments.

In the embryos of the polyploid crucian carp system, expression patterns of the *jnk1b* gene were different from those of the *jnk1a* gene. For example, *jnk1b* expression in the early embryos was higher than in the subsequent stages of embryonic development, where *jnk1b* expression peaked in the early cell-cleavage stage. We also detected strongly upregulated expression of the *jnk1b* gene during brain differentiation in allotetraploid crucian carp, whereas in triploid crucian carp, high expression of *jnk1b* was detected at the stage of muscle development, which was in contrast to *jnk1b* expression observed in the other 3 crucian carp species (Figure 6).

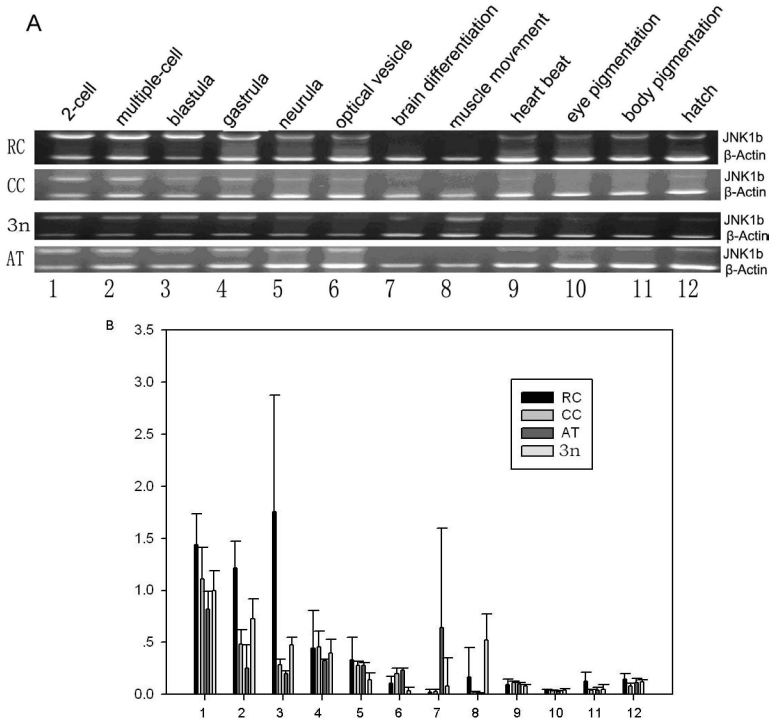

Figure 6. RT-PCR analysis showing the level of jnk1b mRNA in the embryos of allotetraploid crucian carp (AT) and other three fish in the polyploid crucian carp system [triploid crucian carp (3n), common carp (CC) and red crucian carp (RC)] from the stage of cleavage to that of hatching (**A**). Relative level of expression (fold) calculated by dividing the total pixel from each jnk1b mRNA band with the total pixel from the corresponding β-actin mRNA band (**B**). Quantitative results are from three independent experiments.

DISCUSSION

In recent studies, full-length cDNAs for the *jnk1* gene have been cloned in several fish species, including goldfish (GenBank accession No. EU374209) and the rice-field eel (*Monopterus albus*; EF661977). This has revealed a high level of similarity of the *jnk1* gene among these fishes and other vertebrates (Xiao et al., 2010). Here, we have cloned the *jnk1a* and *jnk1b* genes from the polyploid crucian carp system, which includes allotetraploid crucian carp, triploid crucian carp, and their parents, red crucian and common carp. An analysis of the genetic distances of the *jnk1* gene homolog, *jnk1a*, among these different carp species indicated that *jnk1a* maintains a high level of conservation in the polyploid crucian carps (see Figure 2). As shown by the phylogenetic tree constructed from *jnk1a* gene sequences (see Figure 7), triploid crucian carp and its original female parent (red crucian carp) showed a very close phylogenetic relationship, as did allotetraploid crucian and common carp, the original paternal parent of allotetraploid crucian carp. The genetic analysis of the *jnk1a* gene indicated that triploid crucian carp had a closer phylogenetic relationship with its female parent. The *jnk1a* gene analysis also suggested a greater affiliation between allotetraploid crucian carp and its paternal parent.

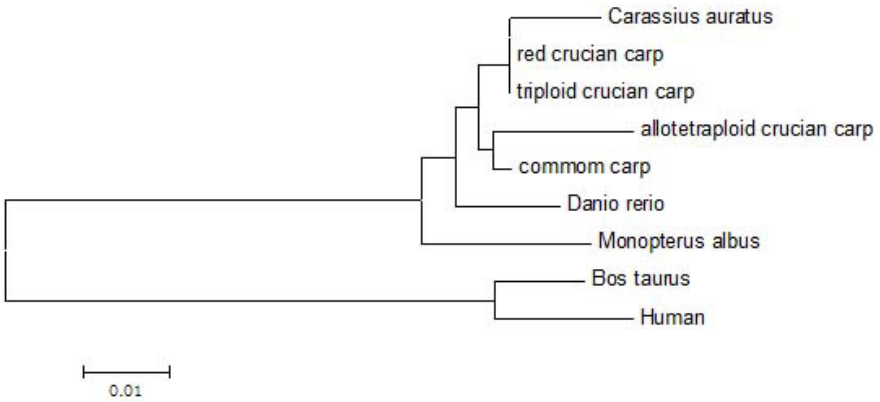

Figure 7. Phylogenetic tree of the *jnk1a* gene in the fish of polyploid crucian carp system (red crucian, common, allotetraploid crucian, and triploid crucian carp) and the *jnk1* gene of other vertebrates (*Carassius auratus*, *Danio rerio*, *Monopterus albus*, *Bos taurus*, and human) from NCBI. This phylogenetic tree was generated through comparative analysis of the *jnk1a* (*jnk1*) coding sequences and using UPGMA calculation and the MEGA3.1 software.

Using the MEGA3.1 software and on the basis of *jnk1b* gene sequences, the genetic distances were determined between allotetraploid crucian, triploid crucian, red crucian, and common carp, and these distances were used to construct a phylogenetic tree by using Neighbor-Joining methods (see Figure 8). In this *jnk1b* gene tree, allotetraploid crucian and the common carp formed a common branch, also shared with triploid crucian carp. Further aggregation with red crucian and Japanese carp led to the formation of a shared clade. These results indicated that the *jnk1b* gene of polyploid crucian carps (i.e., allotetraploid and triploid crucian carp) was more similar to that of their paternal parent than that of the female parent.

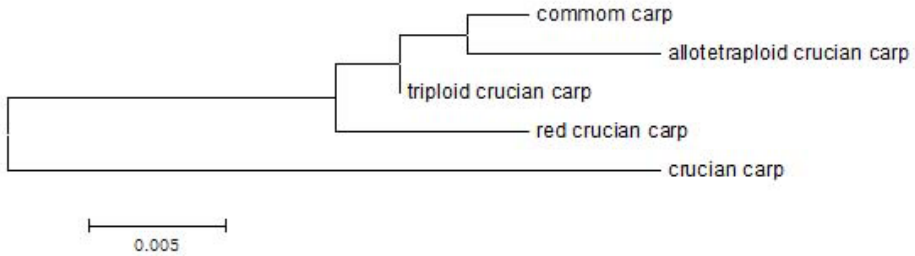

Figure 8. Phylogenetic tree of the *jnk1b* gene in Japanese carp (crucian carp) and in the fish of polyploid crucian carp system (red crucian, common, allotetraploid crucian, and triploid crucian carp). This phylogenetic tree was generated through comparative analysis of the *jnk1b* coding sequences using UPGMA calculation and the MEGA3.1 software.

The MAPK family is a large group of Ser/Thr protein kinases, which are activated by dual phosphorylation of specific Thr and tyrosine (Tyr) residues in a typical "Thr-X-Tyr" motif located within an "activation/phosphorylation loop" (Davis, 1993; Widmann, et al., 1999). As a member of MAPKs, the JNK contains an activation sequence "Thr-Pro-Tyr" (TPY) motif in its kinase domain VIII (Davis, 2000). Three genes, *jnk1*, *jnk2*, and *jnk3,* each encode JNKs

in mammals (Barr and Bogoyevitch, 2001). Comparisons of these JNK proteins in GenBank, showed that the "TPY" motif appeared in different regions of this protein family: the "TPY" motif appeared at amino acid positions 183-185 in the JNK1 protein, 181-183 in JNK2, and at 221- 223 in JNK3. In this study, we cloned 2 genes *jnk1a* and *jnk1b*, from the polyploid crucian carp system. The identity of jnk1a amino acids sequence in the polyploid crucian carps was 95.5%, and the identity of JNK1b amino acids sequence in the polyploid crucian carps 94.3%. A comparison on the basis of the *jnk1a* and the *jnk1b* gene sequence from the polyploid crucian carp system showed that the amino acid sequences of their predicted proteins were about 83% similar. The "activation loop" "TPY" motif was presented at positions 183-185 sites in all amino acid sequences. The predicted amino acid sequences of the *jnk1b* and the *jnk1a* genes showed changes in about 380-427 sites in the polyploid crucian carp system. These results confirmed that the *jnk1a* and *jnk1b* genes identified here represent 2 splice forms arising from the *jnk1* gene in fish.

JNK can phosphorylate a range of substrates owing to its activation by phosphorylation of specific Thr and Tyr within the activation loop. Activated by the upstream kinase MKK4, which phosphorylates Tyr-185 and by MKK7 phosphorylating Thr-183, JNK1 phosphorylates the transcription factor c-Jun on its N-terminal transactivation domain at 2 Ser residues, Ser-63 and Ser-73, to activate AP-1 (Dérijard et al., 1994). Many substrates of JNK are transcription factors, such as Pax2, Elk-1, Foxo4, P53, and ATFII; hence their phosphorylation through JNKs can directly cause changes in gene expression after cells are exposed to a range of cytokines and stress stimuli (Cavigelli et al., 1995; Kawasaki et al., 1996; Mizukami et al., 1997; Chang and Karin, 2001; Tanoue and Nishida, 2003; Fecher et al., 2008). JNK1 also modulates chromatin structure through phosphorylation of histones and regulates different transcription factors (Wolter et al., 2008). In mammals, many diseases are associated with JNK, including eye disease, chronic inflammation (Johnson and Nakamura, 2007), neurodegeneration (Savage et al., 2002), diabetes (Fukuda et al., 2008), and cancer (Uhlirova et al., 2005). The *jnk1* gene is associated with microtubule. *jnk1* gene knockout mice exhibition disrupts the formation of anterior commissure tract and generates a progressive loss of microtubules within axons and dendrites (Chang et al., 2003).

JNK signaling is also involved in controlling oocyte development in other vertebrates (Vigneron et al., 2004; Browaeys-Poly et al., 2005). Our previous studies indicated that the expression of the *jnk1* gene displays distinct patterns during the sex reversal of the rice-field eel *M. albus*, which has some typical features of natural sexual reversal during its lifecycle (Xiao, 1995; Xiao and Liu, 1995; Xiao et al., 2007). In *M. albus*, the *jnk1* gene is highly expressed in the ovary of female individuals, and is substantially decreased at the subsequent intersex stage. However, when the intersex individual develops into the male stage, the expression of JNK1 in the testis is distinctly downregulated (Xiao et al., 2010). In this study, we have demonstrated that both *jnk1a* and *jnk1b* genes show a pattern of wide expression in the embryos and in adult tissues in the polyploid crucian carp system. In comparison with their original parents (red crucian and common carp), distinct expression patterns of *jnk1* in specific tissues or in adult tissues existed in the allotetraploid crucian and the triploid crucian carp. It was evident that the expression level of *jnk1b* was higher in the ovary than that in the testis of polyploid crucian carps, especially in allotetraploid crucian carp. Conversely, few differences existed in the expression levels of the *jnk1a* gene among the polyploid crucian carp system, especially for the triploid crucian carp. Fluctuation of *jnk1* (including *jnk1a* and *jnk1b*) mRNAs during the

development of polyploid crucian carps implies a fundamental role in regulating the developmental process. The different expression patterns of *jnk1* genes in the gonads of polyploid crucian carps also suggest an involvement of the *jnk1* genes in the reproductive development of polyploid hybrid carps, particularly for the fertility of allotetraploid carp and the sterility of triploid carp.

ACKNOWLEDGMENTS

Research supported by the National Basic Research Program of China (#2012CB22305), the National Science Foundation of China (#31172399, #30871908), and the Cooperative Innovation Center of Engineering and New Products for Developmental Biology of Hunan Province.

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