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Elevated Expression of *follistatin* Gene in the Pituitaries of Allotriploid Crucian Carp

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Abstract

As a paracrine factor, Follistatin (Fst) plays an important role in regulating biosynthesis and release of pituitary gonadotropin (GTH), gonadal development, and ovulation cycle. In this study, full-length complementary DNAs (cDNAs) of the *follistatin* gene in diploid red crucian carp, *Carassius auratus* red var., allotriploid crucian carp, and allotetraploid hybrids were obtained. Our data showed that the cDNAs of *follistatin* of all three fish encoded 322 amino acids. Referring to the structural characteristics of Fst in other species, we found that the Fst protein of these three fish had four domains, respectively. Reverse transcriptase polymerase chain reaction (PCR) indicated that the *follistatin* gene was widely expressed in all tested tissues, except spleens, in these three fish. Real-time quantitative PCR analysis of different seasonal profiles showed that allotriploids had significantly higher expression of *follistatin* messenger RNA in pituitaries during both the prespawning and spawning periods. These results suggest that the elevated expression of the *follistatin* gene in the pituitaries of allotriploids might lead to sterility of allotriploids by blocking the inhibitory effect of activin on luteinizing hormone β subunit. Furthermore, the results may improve our understanding of reproduction characteristics in triploids, which benefits polyploidy breeding.

KEYWORDS

allotetraploid, allotriploid, elevated expression, *follistatin* gene, sterility

Fish reproduction is primarily controlled by the hypothalamic–pituitary–gonad (HPG) axis. Gonadotropin-releasing hormone (GnRH), gonadotropin (GTH), and gonadotropin hormone receptor (GTHR) are important signaling molecules in the HPG axis. Regulation of GTH secretion and ovarian development are an important part of fish reproductive endocrine research and are also a high-priority area of research. Follistatin (Fst) is a single-chain glycoprotein hormone (GH). As a paracrine factor, it plays an important role in regulating the biosynthesis and release of pituitary GTH, gonadal development, and the ovulation cycle (Bilezikjian et al. 2004; Cheng et al. 2007; Lin and Ge 2009). Fst can specifically bind to activin with high affinity, neutralizing or blocking the biological effects of the latter in a variety of tissues (Phillips and de Kretser 1998). Meanwhile, Fst also interacts with other members of the transforming growth factor β (TGF-β) superfamily, such as GDF-8/9 and BMP-2/5/7/8, and TGF-β3 (Takahashi et al. 1992; Thompson et al. 2005; Lerch et al. 2007; Zhu et al. 2007). The earlier study of Fst was limited to its role during the reproductive cycle of higher vertebrates (Ueno et al. 1987). Subsequent studies have shown that Fst is present in a variety of tissues including the pituitaries in many animals (DePaolo et al. 1991; Wang et al. 2000; Bilezikjian et al. 2006; Tian et al. 2008; Rajput et al. 2014), suggesting a wide

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range of biological activities at various sites. Fst also plays an important role during embryonic development, which has the function of inducing the differentiation and formation of mesoderm, nerve tissue, and organs (Asashima et al. 1991; Hashimoto et al. 1997; Phillips and de Kretser 1998; Gamer et al. 1999). To date, *follistatin* complementary DNA (cDNA) has been cloned from more than 10 fish species, including goldfish, *Carassius auratus*; zebrafish, *Danio rerio*; grass carp, *Ctenopharyngodon idella*; white catfish, *Ameiurus catus*; largemouth bass, *Micropterus salmoides*; large yellow croaker, *Larimichthys crocea*; tongue sole, *Cynoglossus semilaevis*; and Japanese flounder, *Paralichthys olivaceus* (Bauer et al. 1998; Gregory et al. 2004; Li et al. 2007; Liu et al. 2007, 2014; Wen et al. 2015). In addition, with regard to pituitary, pituitary-derived follistatin exert locally to regulate activin effects on gonadotropes (Bilezikjian et al. 2006). Using cultured goldfish pituitary cells, Yuen and Ge (2004) have demonstrated that recombinant porcine follistatin can counteract the effects of activin on the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) expression in goldfish. Similar effects have been expounded in zebrafish (Lin and Ge 2009). In eel, Aroua et al. (2012) have elaborated that recombinant human follistatin, respectively, antagonized both the stimulatory and inhibitory effects of activin on *FSHβ* and *LHβ* expression using primary cultures of eel pituitary cells. More recently in grass carp, Fung et al. (2017) elucidated that LH but not FSH could suppress *activin* and *follistatin* messenger RNA (mRNA) expression in grass carp pituitary cells in a time- and dose-dependent manner.

In our previous study, fertile diploid females and males were found among F_1 hybrids after mating of female red crucian carp, *C. auratus* red var. ($2n = 100$) with male common carp, *Cyprinus carpio* L. ($2n = 100$), and F_2 diploid hybrids were obtained by self-crossing of F_1 individuals. Interestingly, a portion of the F_2 hybrid females and males were able to produce diploid gametes, respectively, forming tetraploid F_3 hybrids after fertilization ($4n = 200$). Successive breeding produced the F_{26} generation. We also obtained a bisexual fertile allotetraploid population ($4n = 200$, 4*n*AT), which maintained tetraploidy for 24 generations, from F_3 to F_{26} (Liu et al. 2001a, 2001b). The sterile allotriploid crucian carp, which has multiple advantages, such as sterility, faster growth rate, good flesh quality, and higher anti-disease ability, was produced by crossing female Japanese crucian carp, *Carassius cuvieri*, with male 4*n*AT. Although allotriploid crucian carp possess three types of gonads, testis, ovary, and fat type, these organs cannot all produce normal gametes (Liu et al. 2000). Nonetheless, sterile and fast-growing allotriploids are commercially valuable, with the sterile offspring serving as a model for comparative studies in fish reproduction and aquatic application (Long et al. 2006). A preliminary study of the molecular mechanism involved in the sterility of allotriploid fish was performed, and the results showed that meiosis abnormalities and the abnormal expression of *Gnrh*, *Gthβ*, and *Gthr* directly led to the sterility of allotriploid fish (Long et al. 2006; Long et al. 2009).

The gonadal developmental mechanism of vertebrates has been a high-priority research topic in biology for years. The study of *follistatin* related to the gonadal development of teleosts has been widely reported, but few studies in polyploid fish have been reported, and different-ploidy cyprinid fish are good experimental materials for studying the function and mechanism of the *follistatin* gene in polyploid species. In this study, homologous clones and rapid amplification of cDNA ends (RACE) were used to obtain the full-length *follistatin* gene in diploid red crucian carp, allotriploid crucian carp, and allotetraploid hybrids. The expression of *follistatin* mRNA in these three fish was analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) and real-time quantitative PCR (RT-qPCR). The present study provides a theoretical basis for a further understanding of the molecular endocrine mechanism on fertility of allotetraploid and sterility of allotriploid, which is of great significance in fish genetic breeding, especially in polyploidy breeding.

Materials and Methods

Materials

Diploid red crucian carp, allotriploid crucian carp, and allotetraploid hybrids were collected from the Engineering Center of Polyploid Fish Breeding of the National Education Ministry located at Hunan Normal University during the same period (April and October). After the fish were anesthetized, the required tissues were excised and stored at −80 C.

RNA Isolation and Generation of First-strand cDNA

Three samples from each different-ploidy cyprinid fish were randomly selected. Total RNA was isolated from pituitaries, ovaries, testes, kidneys, livers, hearts, spleens, and muscles of different-ploidy fish using the E.Z.N.A.® HP Total RNA Kit (OMEGA, Norcross, GA, USA). The extracted total RNA was subjected to gel electrophoresis to detect the integrity of the fragments, and the concentration was measured with a spectrophotometer. Total RNA with good results was immediately utilized for reverse transcription or stored at −80 C.

First-strand cDNA was synthesized using a RevertAid™ First Strand cDNA Synthesis Kit (Thermo, Wilmington, DE, USA). The reverse transcription product was promptly applied to PCR amplification or stored at −20 C.

Cloning of a Partial Follistatin cDNA Fragment and RACE

Based on the cDNA sequence of the goldfish (AY518590) *follistatin* gene available at NCBI, Primer premier 5.0, and Jellyfish 1.4 were used to design a specific primer complementary to the middle region of the *follistatin* coding region (Table 1). The cDNA fragments of the *follistatin* gene from the three types of fish were amplified by PCR, and the PCR products were detected by electrophoresis through 1.2% agarose gels stained with ethidium bromide. The reaction system was expanded and purified after detection of the target fragment. The recovered DNA fragments were cloned into the pMD18-T vector (Takara, Dalian, China), and this recombinant plasmid was transferred into *Escherichia coli DH5α*. Positive clones, which were identified by blue/white screening and PCR amplification, were sequenced by Sangon (Shanghai, China).

TABLE 1. *Primers used in this study.*

Primer name	Primer sequence (5′–3′)	Usage
F-S	GAGTAAGCCTCGCTGCGTCTG	Clone
F-A	CGCTGGGATATGTGGTGTTGT	Clone
3′-sites Adaptor Primer	CTGATCTAGAGGTACCGGATCC	3′RACE
3R1	GACCAGACAAACAACGCATACTGT	3′RACE
3R2	GGGATTCTAAGATGGGTCGTG	3′RACE
SMART II™ A	AAGCAGTGGTATCAACGCAGAGTACGCGGG	5′RACE
5′-RACE CDS Primer A	(T)25V N (V = A/G/C; N = A/C/G/T)	5′RACE
UPM (Mix)	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT(long) CTAATACGACTCACTATAGGGC(short)	5′RACE
NUP	AAGCAGTGGTATCAACGCAGAGT	5′RACE
5R1	GTTGTTTGTCTGGTCCACCACGCAAG	5′RACE
5R2	CGACCCCTTCCAGGTGATGTTAGAGCAG	5′RACE
F-S1	GAGTAAGCCTCGCTGCGTCTG	RT-PCR
F-A1	CGCTGGGATATGTGGTGTTGT	RT-PCR
F-RS	CGTGCTTGCTTGGTAGATCCA	RT-qPCR
F-RA	TGACTTGGCCTTGATGCATTT	RT-qPCR
β-actin (+)	TCCCTTGCTCCTTCCACCA	Internal control
β-actin (−)	GGAAGGGCCAGACTCATCGTA	Internal control

RACE = rapid amplification of cDNA ends; RT-PCR = reverse transcriptase polymerase chain reaction; RT-qPCR = real-time quantitative polymerase chain reaction.

3′-RACE: The specific 3′-RACE forward primers 3R1 and 3R2 (Table 1) were designed and synthesized according to the known partial cDNAs of different-ploidy fish. The first-strand template was synthesized by a reverse transcription reaction with Oligo dT-3 sites Adapter Primer using 3′-Full RACE Core Set (Takara, Tokyo, Japan). Subsequently, 3R1 and 3′-sites Adaptor Primer were used with the first PCR reaction, and then nested PCR was used for 3R2 and the 3′-sites Adaptor Primer. 5′-RACE: The 5′ ends of *follistatin* cDNAs in these fish were obtained using a SMART™ RACE cDNA Amplification Kit (Clontech, San Francisco, CA, USA). The first-strand cDNA was synthesized by a reverse transcription reaction using SMART II A Oligonucleotide, 5′-RACE CDS Primer A (Table 1), and PowerScript™ Reverse Transcriptase. Similarly, the first PCR was amplified with Universal Primer A Mix (UPM) and 5R1 and the second with Nested Universal Primer A (NUP) and 5R2 (Table 1).

The full-length cDNAs of the three types of fish were obtained by recovering, connecting, transforming, identifying, and sequencing the products of 3′-RACE and 5′-RACE.

RT-PCR and RT-qPCR

The expression of the *follistatin* gene in the pituitaries, ovaries, testes, kidneys, livers, hearts, spleens, and muscles of different-ploidy fish from the spawning season was assayed by RT-PCR. The specific primers (F-S1 and F-A1) were designed against the same coding regions. At the same time, the primers (*β-actin* [+] and *β-actin* [−]) based on the *β-actin* of goldfish (AB039726) were used as the internal control (Table 1). The relative expression of *follistatin* in various tissues of different-ploidy cyprinid fish was determined.

The transcriptional level of the *follistatin* gene (*β-actin* gene was used as the internal control) in the pituitaries of the different-ploidy fish from the prespawning and the spawning seasons was analyzed by RT-qPCR. The specific primers (F-RS and F-RA) were chosen based on the same coding regions in these fish (Table 1). To eliminate unspecific amplification, the most appropriate annealing temperature was determined based on PCR amplification and polyacrylamide gel electrophoresis. PCR reactions and detection were carried out using a Prism 7500 Sequence Detection System (ABI, Foster City, CA, USA), in strict accordance with the operating procedures of the instrument. To ensure the accuracy of the PCR results, sample analysis was repeated thrice. The RT-qPCR program was 50 C for 2 min, 95 C for 10 min, followed by 40 cycles at 95 C for 15 sec and 61 C for 45 sec. According to the $2^{-\!-\Delta\Delta CT}$ method provided by Livak and Schmittgen (2001), the relative expression profiles were obtained by analyzing the relative quantitative results. All data are expressed as the mean value ± SEM. Significant differences were determined using one-way ANOVA from SPSS 19.0 software (IBM, Chicago, IL, USA). Differences were considered significant at $P < 0.05$.

Analysis of Sequence Similarity

The full-length cDNAs of the *follistatin* gene were analyzed using DNASTAR 7.1; the open reading frames (ORFs) were found, and the corresponding amino acid sequences were deduced. The Simple Modular Architecture Research Tool (http://smart.embl-heidelberg.de/) was used to obtain the signal peptide sequence. The protein sequence of Fst in vertebrates was searched in the GenBank database of NCBI. The sequence similarity of amino acid sequences of Fst protein from the diploid red crucian carp, allotriploid crucian carp, allotetraploid hybrids, and other species was analyzed using DNAMAN (version 5.0; Lynnon Biosoft, Quebec, Canada). The Neighbor-Joining tree was constructed with MEGA 5 software (Philadelphia, Pennsylvania).

Results

Cloning and Analysis of Full-Length cDNAs

The intermediate fragments of the *follistatin* gene-coding region of different-ploidy fish, which were obtained by amplification, were sequenced, and the sequences were analyzed by Jellyfish and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The results showed that the fragments were homologous to zebrafish

follistatin and goldfish *follistatin*. The 5′-RACE and 3′-RACE products of *follistatin* from these fishes were respectively sequenced, and the full-length cDNAs were obtained after splicing partial coding regions. As shown in Figure 1, the full-length sequence of *follistatin* in diploid fish was 1243 bp long (KJ093502), but 1250 bp in allotriploids (KJ093503) and 1266 bp in allotetraploids (KJ093504). Sequence analysis revealed that the ORF of these cDNAs was 969 bp long, encoding a protein of 322 amino acids. The deduced ORF amino acid sequences contained a putative signal peptide of 32 amino acids and a mature polypeptide of 290 amino acids. Reference to the structural characteristics of Fst in house mouse (Michel et al. 1990; Nakatani et al. 2002), largemouth bass (Li et al. 2007), and other animals allows division into four domains: N-domain, Domain I, Domain II, and Domain III. The N-domain contained six Cys residues among these three fish. Domain I, Domain II, and Domain III each contained 10 Cys residues in the diploids and allotriploids, while in allotetraploids, Domain I contained nine Cys residues, and Domain II and Domain III each contained 10 Cys residues.

Sequence Similarity Analysis and Construction of a Molecular Evolutionary Tree

In the present study, we compared the amino acid sequences of Fst in different-ploidy fish with other species (Fig. 2). The results indicated that the Fst amino acid sequence had high similarity among fish, especially in several carp species, with similarities of more than 93%. Sequence alignment revealed that the Fst protein of the three different-ploidy fish were highly similar (95.3%), indicating high conservation of function and Fst protein evolution in these fish. However, protein similarities of these three fish with other teleost fish were 87.23–97.20%. These three fish had the lowest similarity (74.53–77.43%) with two types of nonfish vertebrates, which are house mouse and goat, respectively.

Figure 3 illustrates a Neighbor-Joining phylogenetic tree constructed by MEGA 5 software that compares the amino acid sequences of different-ploidy fish and other vertebrates. The results were consistent with the similarity analysis, with red crucian carp and the allotetraploid hybrids forming a common branch. Further aggregation with allotriploid crucian carp, grass carp, and zebrafish led to the formation of a shared clade. All fish were clustered together as a large clade. In contrast, nonfish vertebrates were clustered into a large clade.

Tissue Distribution of follistatin *mRNA by RT-PCR*

Figure 4 shows the tissue distribution of the *follistatin* mRNA from the three different-ploidy fish as determined by RT-PCR, indicating that *follistatin* gene was widely expressed in all tested tissues, except spleens, in these three fish. Compared to other tested tissues, including the hearts, kidneys, livers, and testes, we also found high levels of *follistatin* expression in the pituitaries, ovaries, and muscles of these three fish. Meanwhile, *β-actin*, as an internal control, was detected in all tissues by PCR amplification.

Comparative Expression of Follistatin cDNA in Different-Ploidy Cyprinid Fish

The expression pattern of *follistatin* in the pituitaries of the different-ploidy cyprinid fish from the prespawning and spawning periods was assessed by RT-qPCR. As shown in Figure 5, the expression of *follistatin* differed greatly among the three different-ploidy fish. In both the prespawning and spawning periods, allotriploids showed the high levels of pituitary *follistatin* expression compared with diploid and allotetraploid fish ($P < 0.05$). However, no significant differences in *follistatin* expression were found between diploids and allotetraploids ($P > 0.05$).

Discussion

Our previous studies have shown that allotriploid crucian carp possess three types of gonads: testis, ovary, and fat type (Liu et al. 2000). However, these gonads cannot produce normal gametes (Liu et al. 2000). In addition, the sterility mechanism of sterile allotriploid fish is closely related to endocrine abnormality

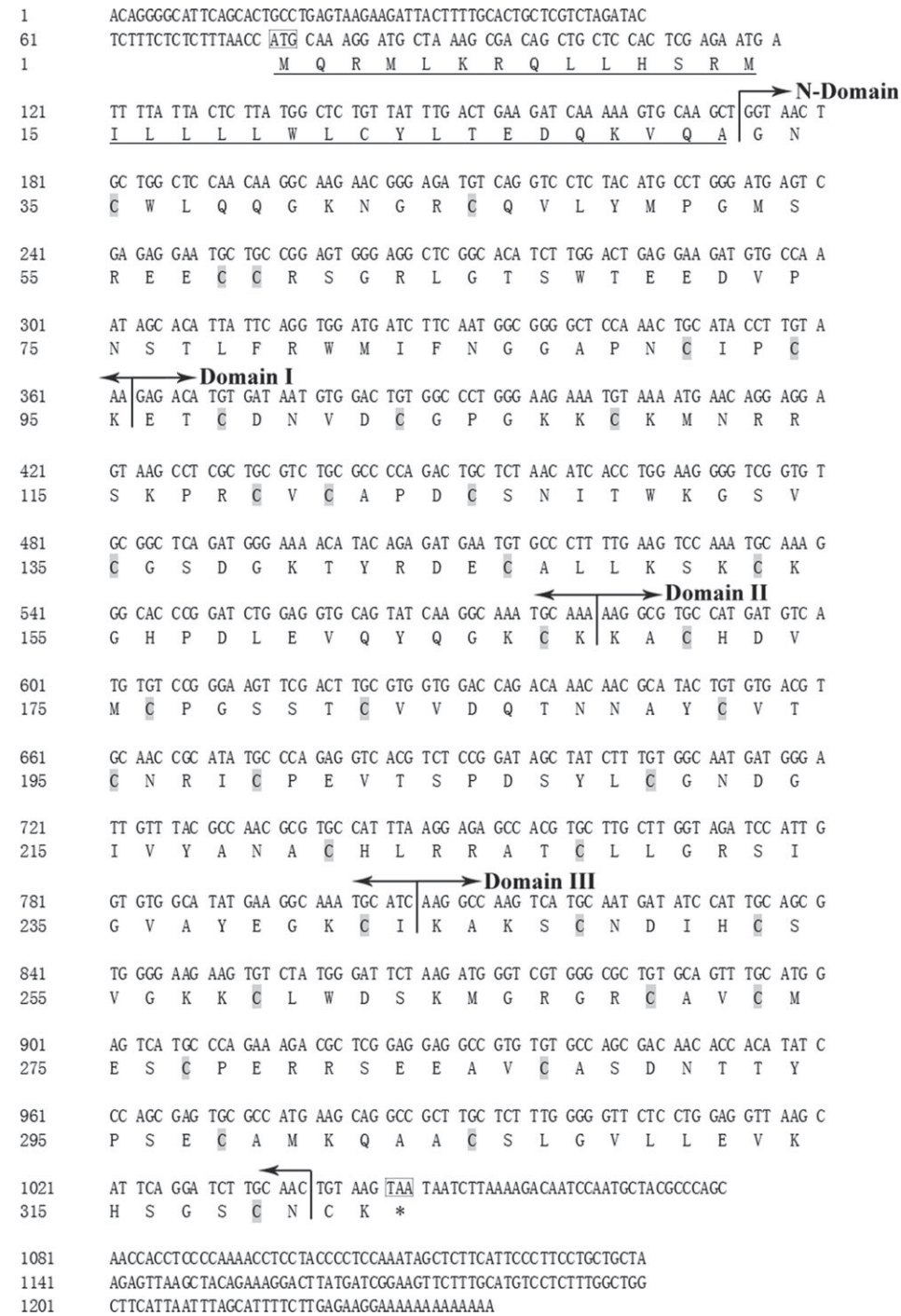

FIGURE 1. *Nucleotide and deduced amino acid sequences of* follistatin *cDNA from different-ploidy cyprinid fish. (A) Diploid red crucian carp; (B) allotriploid crucian carp; and (C) allotetraploid hybrids. The amino acid sequences are shown in the lower row and the nucleotides in the upper row. Nucleotides are numbered from first base at 5′ end. Initiation codons are marked by boxes. Termination codons are marked with asterisks and boxes. Signal peptides are underlined. Cys residues are shaded. Four domains are marked by arrows.*

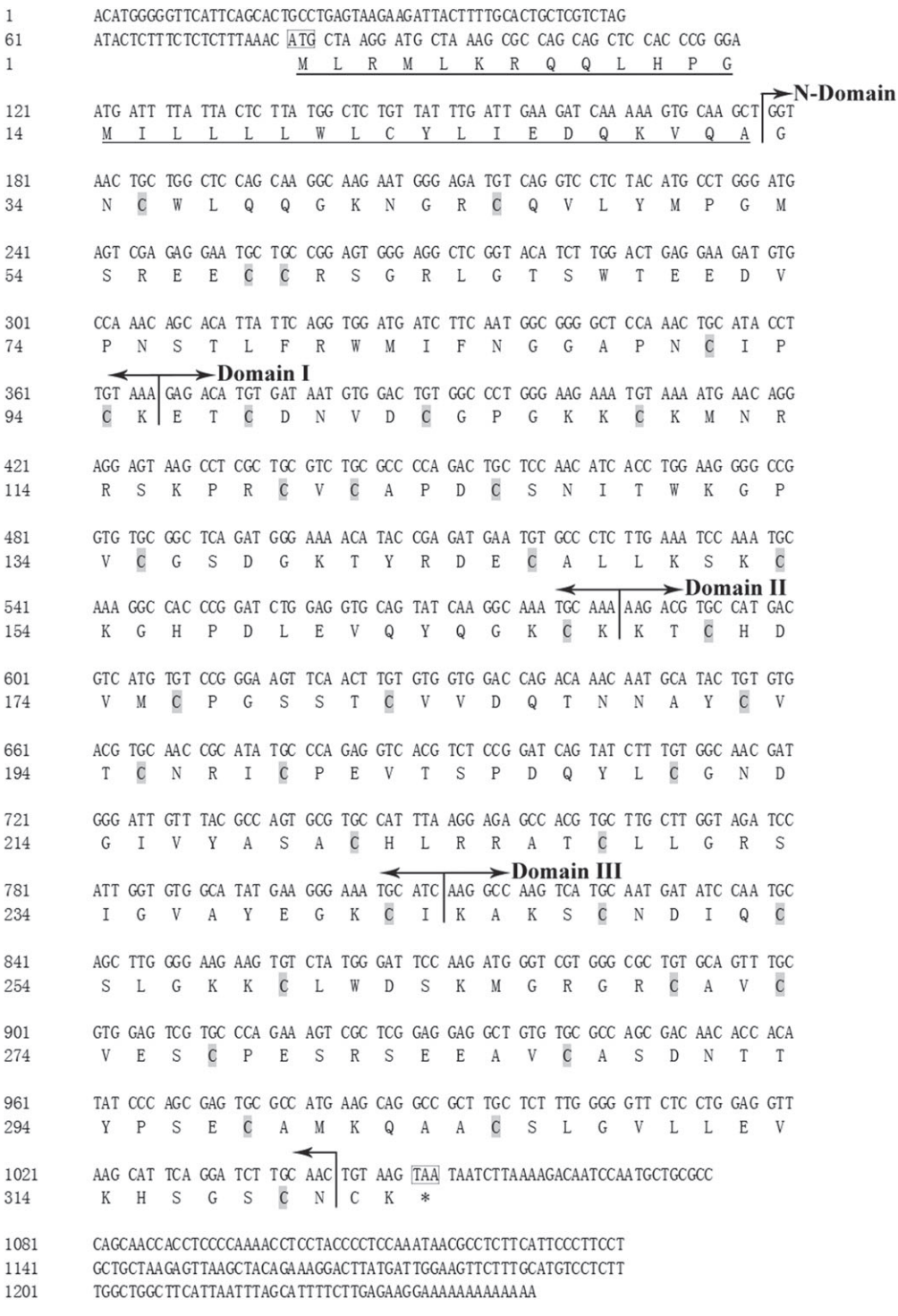

FIGURE 1. *Continued.*

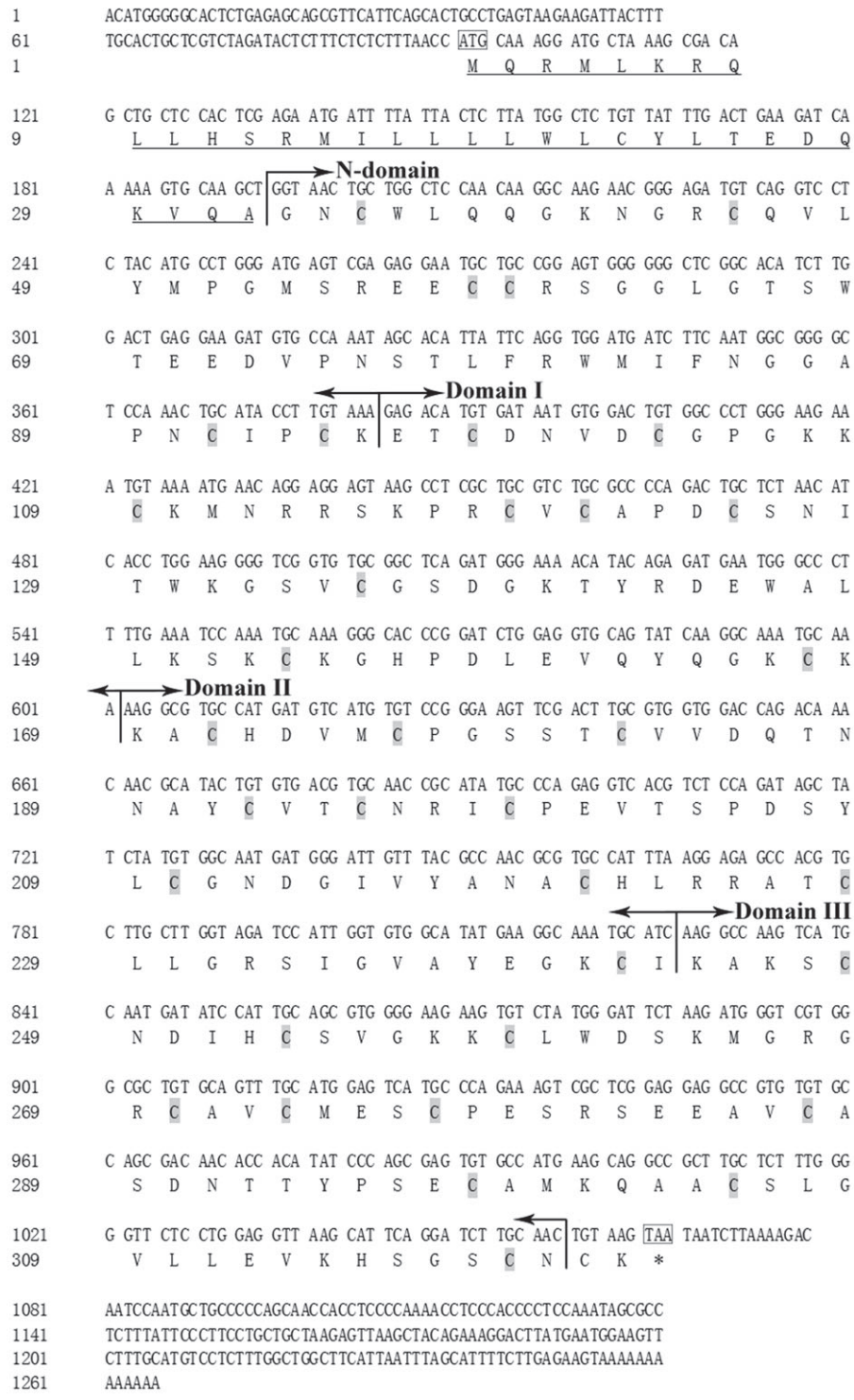

FIGURE 1. *Continued.*

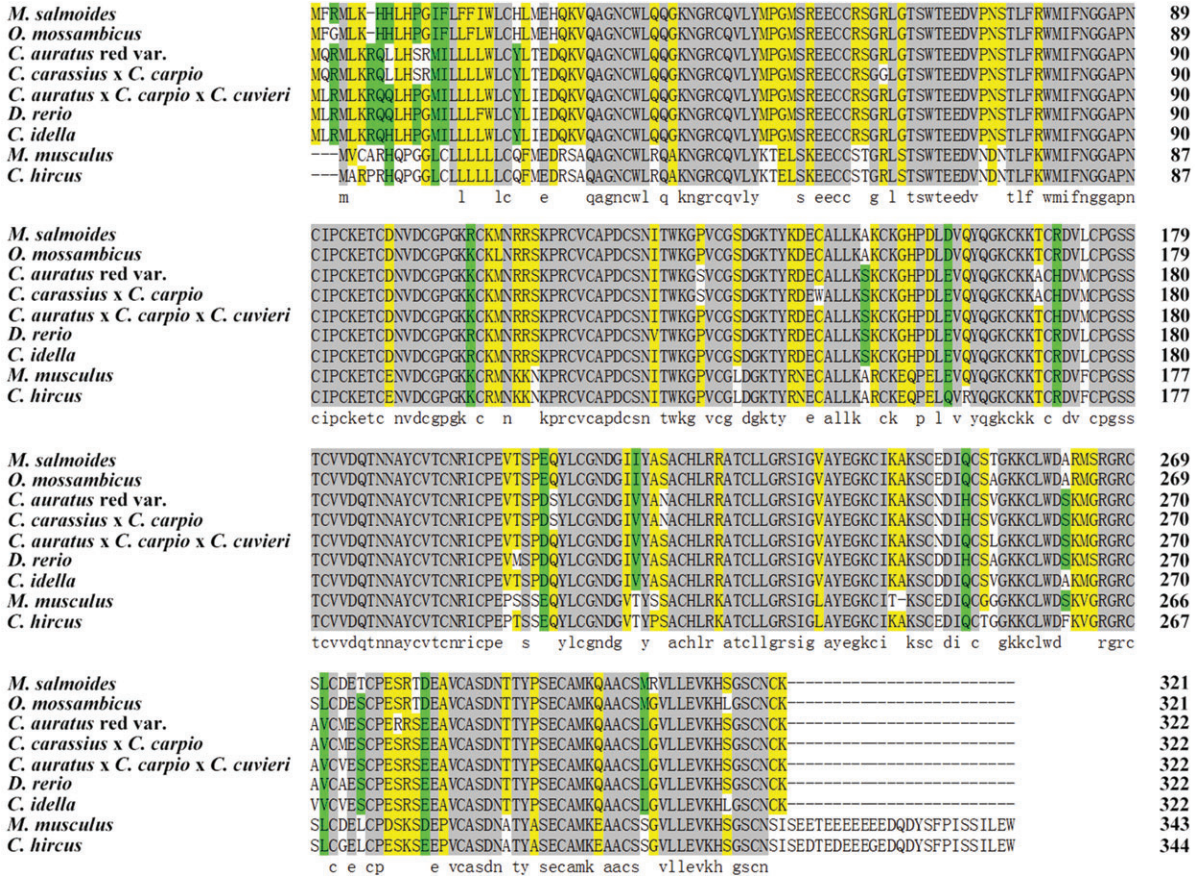

FIGURE 2. *Gaps introduced for best alignment are indicated with points. Identical and similar amino acid residues are indicated with lowercases. Multiple alignment of Follistatin polypeptide sequences of different-ploidy cyprinid fish with corresponding known orthologs. GenBank accession numbers of aligned sequences are as follows: largemouth bass,* Micropterus salmoides *(ABL95955); Mozambique tilapia,* Oreochromis mossambicus *(ABC69147); red crucian carp,* Carassius auratus *red var. (AHN60057); allotetraploid hybrids,* C. auratus × Cyprinus carpio *(KJ093504); allotriploid crucian carp,* C. auratus × C. carpio × Carassius cuvieri *(KJ093503); zebrafish,* Danio rerio *(AAD09175); grass carp,* Ctenopharyngodon idella *(ABC72407); house mouse,* Mus musculus *(NP_001288302); and goat,* Capra hircus *(ADN03390).*

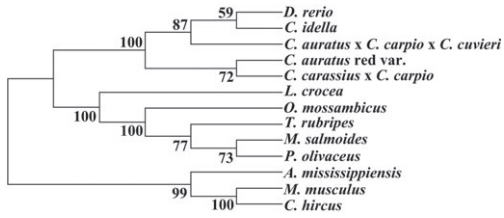

FIGURE 3. *Phylogenetic tree generated by the NJ method based on Follistatin (Fst) sequences in different species. Bootstrap values are indicated at nodes. GenBank accession numbers of Fst are as follows: zebrafish,* Danio rerio *(AAD09175); grass carp,* Ctenopharyngodon idella *(ABC72407); allotriploid crucian carp,* Carassius auratus × Cyprinus carpio × Carassius cuvieri *(KJ093503); red crucian carp,* C. auratus *red var. (AHN60057); allotetraploid hybrids,* C. auratus × Cyprinus carpio *(KJ093504); large yellow croaker,* Larimichthys crocea *(AEC13716); Mozambique tilapia,* Oreochromis mossambicus *(ABC69147); Fugu rubripes,* Takifugu rubripes *(ABC00774); largemouth bass,* Micropterus salmoides *(ABL95955); Japanese flounder,* Paralichthys olivaceus *(ABP04247); American alligator,* Alligator mississippiensis *(AAZ31476); house mouse,* Mus musculus *(NP_001288302); goat,* Capra hircus *(ADN03390).*

(hypothalamic–pituitary–gonad axis) (Long et al. 2006) and reproduction defects (gonadal development) (Liu et al. 2000; Liu et al. 2001a, 2001b). The brain receives the stimuli from the environment to induce the hypothalamus to produce GnRH and gonadotropin release-inhibitory factor, which stimulates or inhibits pituitary synthesis and release of GTH, respectively. GTH then acts on the gonad, causing it to secrete sex steroids to induce gonadal development and maturation (Liu 2010). Research has shown that Fst plays a crucial role in the paracrine networks of GTH expression regulation, which fully illustrates the physiological importance of Fst. Indeed, Fst regulates the synthesis and secretion of FSH, LH, GH, and other hormones in the pituitary via autocrine and paracrine pathways (Bilezikjian et al. 2004).

To further understand the role of Fst in gonadal development of different-ploidy cyprinid fish, we for the first time obtained the entire *follistatin* cDNA sequence of diploid red crucian carp, allotriploid crucian carp, and allotetraploid hybrids. Similarity analysis using DNAMAN 5.0 and BLAST showed Fst amino acid sequence similarity among these three fish of 95.3%. In addition, a Neighbor-Joining tree based on the Fst amino acid sequence of different species showed that five species of cyprinid fish, including red crucian carp, allotriploid crucian carp, allotetraploid hybrids, zebrafish, and grass carp, clustered into a large clade. This finding indicated that the Fst protein was highly conserved in Cyprinidae. However, cyprinids do not exhibit monophyletic evolution in this tree. The origin of red crucian carp is an ancient tetraploid, and the hybrid crucian carps are all polyploid, while zebrafish and grass carp are diploid, which may be why cyprinids are divided into two branches. As the *follistatin* gene in allotriploid crucian carp and allotetraploid hybrids is not completely consistent with its original parents, we speculate that the recombination of chromosome pairing may lead to the differences in *follistatin* between allotriploid and allotetraploid fish and the original parents, which requires further study.

In different-ploidy cyprinid fish, Fst expression in the pituitaries, hearts, kidneys, livers, muscles, ovaries, spleens, and testes was analyzed by

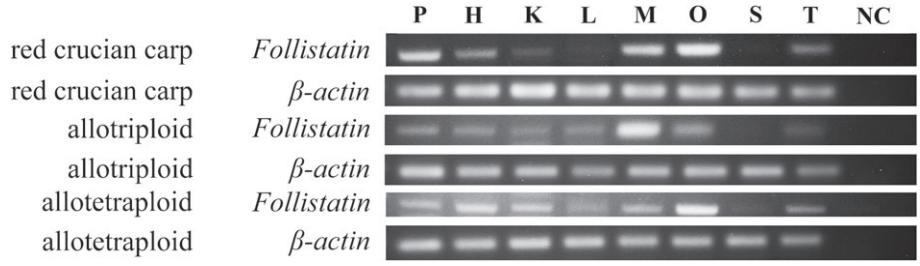

FIGURE 4. *Expression profile of* follistatin *gene in different tissues in three fish.* β-actin *served as an internal control for all samples. H = heart; K = kidney; L = liver; M = muscle; NC = negative control; O = ovary; P = pituitary; S = spleen; T = testis.*

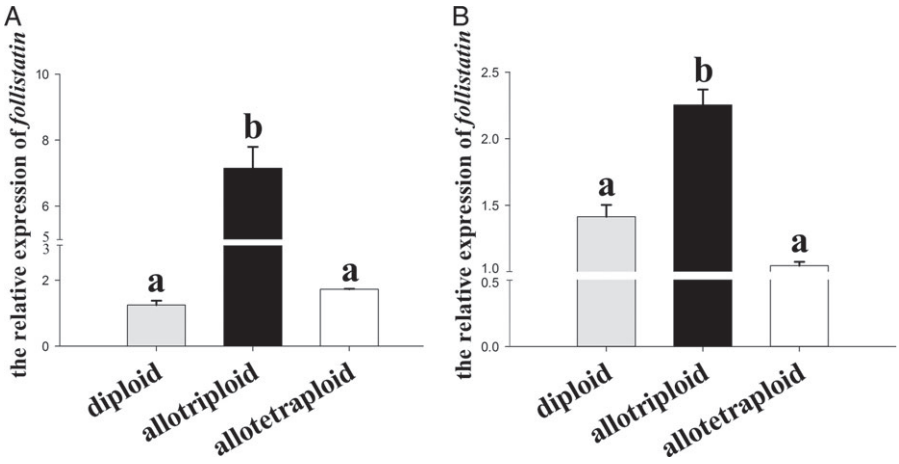

FIGURE 5. *Expression profile of* follistatin *mRNA in the pituitaries of different-ploidy fish. β-*actin *served as an internal control for all samples. (A)* Follistatin *expression in the prespawning period. The data are shown as the mean ± SEM (*n = *3), and values with different letters (a, b) were significantly different between expression levels (*P < *0.05). (B)* Follistatin *expression in the spawning period. The data are shown as the mean ± SEM (*n = *3), and the values with different letters (a, b) differed significantly in expression level (*P < *0.05).*

RT-PCR. The results showed *follistatin* mRNA to be expressed in all these tissues, except the spleens, but there was an apparent difference in expression among the three fish. Fst expression was highest in pituitaries, ovaries, and muscles, indicating a certain role in the processes of gonadal and muscle development in these three fish. Therefore, we speculate that Fst regulates the reproductive activity in these animals and can act via paracrine and autocrine pathways to regulate other members of the TGF-β superfamily (Amthor et al. 2004). Fst also plays an important role in organs and tissues other than the reproductive system, such as the liver, kidney, pancreas, skeletal muscle, and skin (Ying 1989).

Significantly, according to RT-qPCR, *follistatin* mRNA was most highly expressed in the pituitary of allotriploids during both the prespawning and spawning periods compared with diploids and allotetraploids. Allotriploids have indeed abnormality expression in many genes because of haploidy expression. For example, in our previous study, *Dmc1* gene (Tao et al. 2008) and *cyp19a1a* gene (Tao et al. 2014) have been found high expressed in allotriploids. Long et al. (2009) found abnormal expression of *FSHβ* and *LHβ* in allotriploids. Moreover, Chatchaiphan et al. (2017) found a total of 362 transcript-derived unigenes were upregulated and 83 unigenes were downregulated in the triploid bighead catfish, *Clarias macrocephalus* Günther, relative to the diploid bighead catfish by *de novo* transcriptome analysis. Some previous studies have shown that allotriploidy of abnormal expression may be related to the gene expression modification and regulation of the aneuploidy genome (Chen 2013; Matos et al. 2015), which may explain the higher expression of *follistatin* in allotriploids compared with diploids and allotetraploids. A regulatory effect of the activin/follistatin system on *LHβ* expression was previously described in cyprinids. In goldfish, treatment of pituitary cells by recombinant goldfish activin B or recombinant human activin A induces a decrease in *LHβ* mRNA levels (Yuen and Ge 2004). Similar results were recently obtained in zebrafish after treatment of pituitary cells with recombinant goldfish activin B (Lin and Ge 2009). Fst also significantly regulated *LHβ* mRNA levels and was able to counteract the inhibitory effect of activin on *LHβ* in the goldfish pituitary (Yuen and Ge 2004). Moreover, Long et al. (2009) found higher expression of *LHβ* mRNA in allotriploids during the spawning period compared with diploids and allotetraploids. Observation of the pituitary ultrastructure in

different ploidy fish demonstrated that secretory granules and globules in GTH-producing cells of allotriploids are not expelled as they are in diploids and allotetraploids in the spawning season (Long et al. 2006). In addition, expression of *LHβ* mRNA in allotriploids does not decrease normally, which is consistent with its morphological traits. We speculate that the elevated expression of the *follistatin* gene in the pituitary of allotriploids might stimulate the overexpression of *LHβ* mRNA in allotriploids by blocking the inhibitory effect of activin on LHβ, which might further lead to their sterility. Meanwhile, these obtained data would contribute to further study of the molecular mechanism on gonadal development and reproduction in allotriploids, which enhances strategies of triploid breeding.

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