Molecular Characterization of *Myostatin* Gene from Zhikong scallop *Chlamys farreri* (Jones et Preston 1904)

Xiaoli Hu¹, Huihui Guo¹, Yan He¹, Shan Wang¹, Lingling Zhang², Shi Wang², Xiaoting Huang¹, Scott William Roy³, Wei Lu¹, Jingjie Hu¹* and Zhenmin Bao¹

¹Key Laboratory of Marine Genetics and Breeding (Ocean University of China), Ministry of Education, Qingdao 266003, China
²Waggoner Center for Alcohol and Addiction Research, University of Texas at Austin, 1 University Station C0930, Austin, TX 78712, USA
³Department of Biology, Stanford University, Stanford, CA 94305, USA

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The scallop is an economically important sea food prized for its large and delicious adductor muscle. Studying the molecular basis of scallop muscle growth is important for both scallop breeding and our understanding of muscle mass regulation in bivalve. Myostatin (MSTN) is a conserved negative regulator of muscle growth and development. Here we report the MSTN gene from Zhikong scallop (*Chlamys farreri* Jones et Preston 1904). The *C. farreri* MSTN consists of 11651 nucleotides encoding 457 amino acids. The gene has a 3-exon/2-intron structure that is conserved with vertebrate homologs. The exons are 586, 380 and 408 bp in length, respectively, and separated by introns of 5086 and 1518 bp. The protein sequence contains characteristic conserved residues including a cleavage motif of proteolysis (RXXR) and nine cysteines. Three transcription initiation sites were found at 62, 146, and 296 bp upstream of the translation start codon ATG. In silico analysis of the promoter region identified a TATA-box and several muscle-specific regulatory elements including COMP, MEF2s, MTBFs and E-boxes. Minisatellite DNA was found in intron 1. By fluorescence in situ hybridization (FISH), the gene was mapped to the long arm of a pair of middle subtelo-centric chromosome. Quantitative analysis of MSTN transcripts in embryos/larvae indicated high expression level in gastrulae and limited expression at other stages. In adult scallops, MSTN is predominantly expressed in striated muscle, with different expression levels in other tissues. Our data provide valuable genomic and expression information which will aid the further study on scallop MSTN function and MSTN evolution.

**Key words:** *Chlamys farreri*, cloning, mapping, myostatin, quantitative expression

INTRODUCTION

Compared with other bivalve members such as mussel, oyster and clam, scallop has an extraordinary adductor muscle which accounts for about 20%–40% of the total soft mass. The huge and delicious adductor muscle has made scallop a famous seafood consumed the world over. As an important aquaculture species in China, scallop contributes about 1 million metric tons of production per year. The desire to select strains with rapid growth – particular fast muscle growth – for rapid improvement of scallop production has encouraged the studies of the molecular basis of scallop muscle growth. Identification of genes with putative roles in muscle growth could benefit both scallop breeding and our understanding of muscle mass differences in bivalve.

Myostatin (MSTN or GDF-8), which is a member of the transforming growth factor-β (TGF-β) superfamily, acts as a negative regulator of skeletal muscle development...
and growth in mammals (Lee, 2004). Since its initial discovery in 1997, increased muscle mass due to MSTN mutations has been reported in mice (McPherron et al., 1997), cattle (McPherron and Lee, 1997), dog (Mosher et al., 2007), and human (Schuelke et al., 2004). The enhanced muscle growth results from increases of both cell number and cell size (Thomas et al., 2000; Rios et al., 2001; Langley et al., 2002, 2004; McCroskery et al., 2003). For its key role in controlling muscle mass, MSTN has been an important locus for clinical treatment of muscle growth disorders and genetic improvement of farmed animals (Rodgers and Garikipati, 2008; Joulia-Ekaza and Cabello, 2007; Bellinge et al., 2005).

So far, MSTN has been cloned and identified from a wide variety of vertebrates including mammals, birds and fish (Rodgers and Garikipati, 2008). All the characterized vertebrate MSTNs are organized into three exons and two introns, with well evolutionarily conserved amino acid sequences (Rodgers and Garikipati, 2008). In mammals, MSTN is almost exclusively expressed in developing and adult skeletal muscle, and accordingly, physiological functions of MSTNs are essentially confined to skeletal muscle growth and development regulation (McPherron et al., 1997; Kambadur et al., 1997; Carlson et al., 1999). Low expression levels of MSTN were also detected in mammary gland (Ji et al., 1998) and heart (Sharma et al., 1999). In non-mammalian vertebrates, MSTN is expressed more widely, with transcripts found in many different developing tissues of chicken (Castelhano-Barbosa et al., 2005; Kocamis et al., 1999), and almost ubiquitously in tissues of fish (Maccatrozzo et al., 2001; Radaelli et al., 2003). For invertebrates, MSTN homologs have only been characterized from Drosophila melanogaster (Lo and Frasch, 1999), bay scallop Argopecten irradians (Kim et al., 2004) and land crab Gecarcinus lateralis (Covi et al., 2008). Broad tissue distribution of MSTN was also detected in these species, suggesting involvement in several cellular functions. However, giant skeletal muscle phenotype and increased myofibers number due to MSTN inhibition were reported in zebrafish (Xu et al., 2003; Acosta et al., 2005). And for invertebrate, high MSTN expression level was present in skeletal muscle (Lo and Frasch, 1999; Kim et al., 2004; Covi et al., 2008), and apparent functional equivalence in receptor recognition between fruit fly and mammalian MSTNs was found (Lee-Hoeflich et al., 2005). These results suggest the conserved function of MSTN in regulating muscle growth among non-mammalian species.

Although cDNA sequences of bay scallop and land crab MSTNs have been obtained and their expression in adult tissues has been examined, it is not clear whether these invertebrate homologs are organized as in vertebrate or not. Meanwhile, in mollusca, muscle was reported to first appear at the early trochophore stage (Wanninger et al., 1999; Wanninger and Haszprunar, 2002a, b) and a specific well-organized musculature is formed at veliger stage (Odintsova et al., 2006). Investigation of MSTN expression during these development stages could provide us information which is helpful to understand the gene function. Here, we reported the MSTN gene from Chinese native species Zhikong scallop (Chlamys farreri Jones et Preston 1904), including its genomic sequence and organization, chromosomal localization, and quantitative expression profiles in embryos/larvae and adult tissues. In addition to its possible application in the genetic improvement of Zhikong scallop, our findings will also provide valuable information for evolutionary and functional studies of MSTN.

MATERIALS AND METHODS

Sample collection Adult Zhikong scallops were collected from the hatchery of Xunshan Group, Shandong, China. Mantle, gill, gonad, kidney, striated muscle, smooth muscle and digestive gland were dissected, immediately preserved in liquid nitrogen and subsequently stored at –80°C. Fertilized eggs, 2–4 cells, blastula, gastrulae, D-shaped larvae and veliger larvae of C. farreri were collected and preserved as above.

Cloning and characterization of C. farreri MSTN
Total RNAs were extracted from striated muscle with the method described by Hu et al. (2006). Two degenerate primers, MSTNf1 and MSTNr1, corresponding to the conserved amino acid sequences DFVAFGWD and CCTPTKMS, respectively, were used to amplify MSTN fragments (Table 1). The cycle parameters were as follows: an initial denaturation at 94°C for 2 min, 30 cycles of 94°C for 30 s, 57°C for 30 s and 72°C for 30 s. The PCR products of 200 bp were purified, cloned and sequenced. To obtain full-length cDNA, 3′ and 5′ rapid

| Table 1. Summary of primers used in this study |
|-----------------|-----------------|-----------------|
| Primer Name     | Orientation     | Sequence (5′ to 3′) |
| MSTNf1          | sense           | GAYTTYGHTGMITTGTGGTGGA |
| MSTNr1          | antisense       | GACATTTTIGTTGGGGMTCAGCA |
| MSTNf2          | sense           | TCCGTTTGGGTTGGGACTTTGTGATAG |
| MSTNr2          | antisense       | GTGGCGGCGCTTAGTGGGCGCAGC |
| MSTNf3          | sense           | GCCTGTCTGTEGTTTATAGT |
| MSTNr3          | antisense       | CGTTTCTTCTCCTCAGGCT |
| MSTNf4          | sense           | TACCGGAGTTGACCTTGGGACG |
| MSTNr4          | antisense       | TGGAAAAACCTGTTTTAATTGCTT |
| MSTNf5          | antisense       | TCGTTTGGTTGTTGATCGCTACAGCA |
| Actin s         | sense           | TACAAATTTGGGACGATATGG |
| Actin r         | antisense       | ACCGATCCAGACTGAGATTTCC |
amplification of cDNA ends (RACE) were performed, and SMART™ RACE cDNA Amplification Kits (TaKaRa) were employed according to the manufacturer’s instruction. Specific primers MSTNf2 and MSTNr2 based on the sequence of the initial 200 bp gene fragment were designed for 3’ RACE and 5’ RACE, respectively. The full-length cDNA sequence was determined by piecing together the sequences of 3’ and 5’ RACE products.

To get the genomic sequence, *C. farreri* fosmid library (Zhang et al., 2007) was screened by PCR with primers MSTNf1 and MSTNr1, and a clone generating expected amplification product was used to obtain the full length of *MSTN*. Specifically, fosmid DNA was isolated from this clone by standard laboratory methods (Sambrook et al., 1989) and partially digested with restriction enzyme Sau3AI (New England Biolabs). Fragments larger than 2000 bp were collected and purified. PUC19 DNA (TaKaRa) was completely digested with restriction enzyme BamHI (New England Biolabs), purified and incubated with calf intestine alkaline phosphorase (TaKaRa) to remove the 5’ phosphate groups. The fosmid fragments and prepared PUC19 DNA was ligated and transfomed to competent cells (JM109). Three round screening of the sub-clones was continuously performed by PCR with two primers used for each round (MSTNf2-MSTNr2, MSTNf3-MSTNr3 and MSTNf4-MSTNr4, respectively) (Table 1). In the piece of sequence of three overlapping fragments, promoter regions and partial first exon were absent. Then the absent sequence was cloned by genome walking using the Universal Genome Walker kit (TaKaRa). Nested PCR was performed with primers MSTNr4 and MSTNr5 according to the manufacturer’s protocol. The full-length sequence of *C. farreri MSTN* was obtained by merging the sequences from fosmid subclones and genome walking.

Sequences were analyzed by NCBI Blast programs (http://www.ncbi.nlm.nih.gov/blast/) for similarity to known genes and the genomic organization was analyzed by comparing the genomic sequence and cDNA sequence. Multiple alignments of the deduced amino acid sequences were performed using the programs of MegAlign 5.01 (DNASTAR Inc.). The neighbor-Joining method in MEGA version 4.1 was used to construct the phylogenetic tree based on protein sequences. Putative regulatory elements were identified using MatInspector software (Genomatix Inc., www.genomatix.de) which searches consensus sequences of known cis-regulatory sequences. Tandem repeats finder (TRF) software (Benson, 1999) was used to search tandem repeat sequences.

**Chromosomal localization of *C. farreri MSTN***

Chromosome and C0t-1 DNA of *C. farreri* were prepared by the method described by Huang et al. (2006) and Zhang et al. (2008), respectively. Fluorescence in situ hybridization (FISH) was performed according to Zhang et al. (2008). Briefly, the fosmid DNA containing MSTN was labeled with digoxigenin-11-dUTP by nick translation following the manufacturer’s instruction (Roche). Chromosome spreads were pretreated, denatured and dehydrated. Hybridization was carried out by incubating the slides with hybridization mix (10 ng/μl probes, 10% dextran sulfate, and 50% deionized formamide in 2× SSC) and C0t-1 DNA (50 ng/μl) for 16 h at 37°C in a moist chamber. After enough washes, probes were detected with anti-digoxigenin-rhodamine (Roche) and chromosomes were then counterstained with 4’, 6-diamidino-2-phenylindole (DAPI) in antifade solution (Vector Laboratories). Slides were observed using a Nikon Eclipse-600 epifluorescence microscope equipped with a CCD camera. The signals were collected using appropriate filter sets and LUCIA software (Laboratory Imaging).

**Quantitative expression analysis of *C. farreri MSTN***

Real-time RT-PCR was performed to analyze the mRNA expression of *C. farreri MSTN* at different developmental stages (fertilized eggs, 2–4 cells, blastula, gastrulae, D-shaped larvae and veliger larvae, n > 500) and in different adult tissues (striated muscle, smooth muscle, digestive gland, kidney, gill and mantle, n = 3) according to Wang et al. (2008). First-strand cDNA was synthesized from 2 μg total RNA using oligo (dT)18 and MMLV reverse transcriptase (Promega) in a total volume of 25 μl. A control reaction without reverse transcriptase was performed to preclude any DNA contamination. Primers MSTNf4 and MSTNr4 were used to amplify partial fragment of the first exon. *C. farreri* β-actin fragments were amplified with primers Actin s and Actin r. The relative quantity of *MSTN* mRNA was determined by dividing the quantity of *MSTN* by the quantity of β-actin. PCR products of *MSTN* and β-actin were respectively cloned. Plasmids of positive clones were then isolated using a CASpure Plasmid Isolation Kit (CASarray) and the concentrations were determined by an Ultrospec 2100 pro spectrophotometer (Amersham Biosciences). The standard curves of *MSTN* and β-actin were constructed using different copy numbers of plasmids (10^1, 10^2, 10^3 and 10^6) as templates for PCR amplification. All real-time amplification reactions were run on an FTC-2000 Thermal Cycler (Funglyn Biotech) using Sybr green dye included in the 2× Realtime PCR Master Mix (TOYOBO). PCR programs began with initial denaturation at 94°C for 3 min, followed by 45 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s (MSTN) or 1 min (β-actin), and final extension at 72°C for 7 min. Each PCR reaction was performed in duplicate. To exclude the possibility that non-specific products exist in the PCR products, dissociation analysis was performed by subjecting the samples to a constant decrease in temperature (from 95°C to 55°C) while recording fluorescence every 25 s.
Fig. 1. Alignment of MSTNs from human (*H. sapiens*, AAB86694), zebrafish (*D. rerio*, NP_571094), land crab (*G. lateralis*, ACB98643), bay scallop (*A. irradians*, AAT36326) and Zhikong scallop (*C. farreri*, ABJ09581). Identical amino acids are shaded and the proteolytic processing site (RXXR) is boxed. Nine conserved cysteine residues are boxed and denoted with stars. Exon boundaries in MSTN genes of human, zebrafish and Zhikong scallop are indicated by arrows.
RESULTS

Sequence characterization and analysis To get the full cDNA sequence of *Cyprasia farreri* MSTN gene, 3’ and 5’ RACE was carried out. One band was obtained by 3’ RACE, and three bands were obtained by 5’ RACE, which indicate three transcription start sites in the gene. A 3604 bp cDNA sequence was obtained by piecing the 3’ RACE sequence and the longest 5’ RACE sequence. It contains a 1374 bp open reading frame (ORF) which encoded 457 amino acids. Blast analysis showed that the deduced amino acid sequence is most similar to MSTN. As with other TGF-β superfamily members, the *C. farreri* MSTN (GenBank accession No. ABJ09581) possesses a potential proteolytic processing site RXXR (matching with RSKR, residues 337–340). Cleavage at this site would generate a propeptide and a mature peptide. Nine cysteine residues were present in the carboxy-terminus portion which corresponds to the mature processed protein (Daopin et al., 1992). The carboxy-terminal showed higher similarities and the mature peptide of *C. farreri* MSTN exhibited 45%, 45%, 45%, 39.1% and 97.4% identities with those of human, chicken, zebrafish, land crab and bay scallop, respectively.

To determine the relationship of scallop MSTN genes to other TGF-β ligands genes, we performed phylogenetic analysis of land crab and scallop MSTNs along with a variety of human homologs (Fig. 2). A variety of methods each yielded a clade containing MSTNs from scallop, land crab, and human, as well as GDF11 from human; when added, zebrafish MSTN and GDF11 genes each grouped with their human orthologs, as expected. Vertebrate MSTN/GDF11 formed a clade, consistent with MSTN and GDF11 being related by a gene duplication in vertebrate ancestors (Xing et al., 2007). The finding of a MSTN/ GDF11 clade supports the *C. farreri* MSTN gene being orthologous to MSTNs from previously reported species (Kim et al., 2004; Covi et al., 2008).

The genomic sequence generated from fosmid subclones and genome walking was 11651 bp (GenBank accession No. DQ988329), including 1421 bp 5’ flanking region and 2144 bp 3’ flanking region (Fig. 3). 5’RACE results showed three transcription initiation sites which are at 62, 146 and 296 bp upstream of the translation start codon ATG, respectively. Two putative polyadenylation signals (AATAAA) were found at 1055 and 1949 bp downstream from the translation stop codon (TAA), respectively. Their presence in both the 3’ end of genomic sequence and 3’ untranslated region (UTR) indicates that both sites are used for polyadenylation.

Genomic organization As with its vertebrate homologs (Rodgers and Garikipati, 2008), the *C. farreri* MSTN gene is organized into three exons and two introns. The three exons are 586, 380 and 408 bp in length, respectively, and are separated by two introns of 5086 and 1518 bp. All the intron-exon boundaries conform to the GT-AG rule (Sharp, 1981). Like MSTNs from vertebrate (Garikipati et al., 2006), intron 1 is classed as type 2 as it interrupts the codon between the second and third nucleotide and intron 2 is classed as type 0 as it interrupts the coding sequence after one codon (Fig. 3) (Sharp, 1981). Amino acid sequence alignment showed that the intron positions were also well conserved with vertebrates (see Fig. 1; intron positions for the scallop *A. irradians* and the land crab *G. lateralis* are unknown since genomic sequence is not available). Minisatellite DNA with unit length of 157 bp and repeat number of 3.3 was found in intron 1 (Fig. 3). The alignment of different repeat units is shown in Fig. 4.

Promoter analysis Using the program MatInspector, a putative TATA box and several putative muscle-specific transcriptional factor binding sites or cis-regulatory elements were identified. These regulatory elements
Fig. 3. Continued

Fig. 3. Genomic sequence of the *C. farreri* MSTN gene and deduced amino acids. The coding sequences are indicated by capital letters and deduced amino acids are represented by single-letter code. The non-coding sequences are shown in lower case letters. All the exon/intron boundaries have the consensus GT/AT ends. The putative promoter elements are grey-highlighted and represented with names. Nucleotide of each transcription start sites is underlined and denoted by bold letter. Minisatellite sequence in intron 1 and two putative poly (A) signals in the 3' UTR are underlined. The proteolytic processing site (RSKR) and nine conserved cysteine residues are boxed. Stop codon 'TAA' at the end of ORF is marked with an asterisk.
include one COMP (factors which cooperate with myogenic proteins), three myocyte enhancer factor 2 binding sites (MEF2), and four muscle-specific Mt binding sites (MTBF). In addition, three putative E boxes (CAXXTG, \( X = \) any amino acid) which are the binding sites for the basic helix-loop-helix myogenic regulatory factors (MRFs) were also found in the 5’-flanking region (Fig. 3).

**Chromosomal localization** Physical location of the *C. farreri* MSTN was carried out using FISH. As shown in Fig. 5, the fosmid clone carrying MSTN produced clear and unique signals on the *C. farreri* metaphase spreads. The gene was mapped to the long arm of a pair of middle subtelocentric chromosomes, about two-fifth of the long arm away from the centromere.

**Quantitative expression analysis** Expression of MSTN in embryos/larvae and tissues of adult *C. farreri* is shown in Fig. 6. The transcript was undetectable in fertilized eggs, and barely detected from 2–4 cell stage to...
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blastula. After that, the expression level rose immediately in gastrulae, about 200-fold higher than that in blastula, but then greatly dropped at D-shaped larvae stage and afterward subsides to barely detectable level in veliger. In adult tissues, the highest level of MSTN mRNA was observed in striated muscle, and the lowest expression was detected in smooth muscle and digestive gland. Other tissues including mantle, gill, gonad and kidney present intermediate expression.

DISCUSSION

In this study, we characterized the *C. farreri* MSTN gene by analyzing its genomic organization, protein sequence, chromosomal localization, and tissue and embryos/larvae expression. Like all of the characterized vertebrate homologs, *C. farreri* MSTN has three exons and two introns. In addition, the intron-exon boundaries and the location of introns are also well conserved between scallop and vertebrate MSTN genes (Garikipati et al., 2006), indicating the highly conserved genomic structure and pre-mRNA splice sites of MSTN. Mini-satellite DNA with unit length of 157 bp was found in the first intron. A preliminary analysis revealed that it was polymorphic in both the repeat number and the repeat unit sequences, making it a potential marker associated with scallop muscle mass traits. In vertebrate MSTNs, another type of DNA repeat, microsatellites (SSRs) with repeat unit of 2–4 bp were widely found (Xue et al., 2006; Tellgren-Roth et al., 2002; Kocabas et al., 2002; Maccatrozzo et al., 2002). However, no SSR sequence is present in *C. farreri* MSTN and this is the first report of mini-satellite DNA in *MSTN*.

Comparing with its vertebrate homologs, the *C. farreri* MSTN is highly conserved around the carboxy-terminal bioactive domain where conserved cysteine residues and RXXR proteolytic processing site are present. Selective pressures are likely to be responsible for the coding sequence as well as the overall genomic organization. Several insertions in scallops and land crab MSTNs are found in both the amino-terminal and carboxy-terminal regions by alignment with vertebrate MSTNs (Fig. 1). This raises the question of the possibility that the insertions may be related to functional variations of MSTN between vertebrate and invertebrate.

Three transcription start sites were found in the 5' UTR of *C. farreri* MSTN. Previous studies indicated three transcription start sites in the human MSTN, too, but only one in the bovine gene (Jeunplong et al., 2001; Gonzalez-Cadavid et al., 1998). Subsequent analysis of the promoter regions identified several putative cis-regulatory elements, including E-box (CANNTG), MEF2, MTBF and COMP, which have been shown to be required for muscle specific gene transcription. Of particular interest was the presence of multiple E boxes and MEF2 sites. The E-box is one of the sequence motifs for the basic helix-loop-helix myogenic regulatory factors (MRFs) (Apone and Hauschka, 1995; Catala et al., 1995; Ceccarelli et al., 1999). Many muscle-specific genes have multiple E-boxes in their promoter region to cooperatively regulate gene transcription (Rao et al., 1996). As the E-boxes with similar core sequencess play roles to a different extent (Spiller et al., 2002; Du et al., 2007), further studies should be performed to find out the most important ones regulating *C. farreri* MSTN expression. The MEF2 enhancer is critical to the differentiation of skeletal muscle and the induction of MSTN expression (Olson et al., 1995; Ma et al., 2001). In muscle cells, cooperative interaction between MEF2 factors and myogenic basic-helix-loop-helix (bHLH) factors has been shown to regulate muscle-specific transcription (Molkentin et al., 1995). The highest level of MSTN transcript detected in *C. farreri* striated muscle and early muscle development stages implies that these regulatory motifs may play important roles in the regulation of scallop MSTN expression and, therefore, on muscular development. Meanwhile, the ubiquity of MSTN expression in scallop tissues, however, suggests that additional elements unrelated to myogenesis may be active as well.

Physical mapping developed from fosmid clone indicated that *C. farreri* MSTN lies on the long arm of a pair of middle subteloentric chromosomes. As the most important gene regulating muscle mass, MSTN has been mapped on the chromosomes of several mammals, chicken...
and fish by marker linkage or FISH analysis (Rodgers and Garikipati, 2008). In bovine, FISH data in combination with linkage analysis showed that MSTN was located at the muscular hypertrophy (mh) locus (Smith et al., 1997), making MSTN a candidate gene causing the mh. This was further confirmed by finding the mutations in MSTN which are responsible for double muscling phenotype (McPherron and Lee, 1997). In C. farreri, genetic linkage maps has been constructed (Wang et al., 2004; Zhan et al., 2009) and chromosome identification by FISH mapping is undergoing (Zhang et al., 2007). Combining the physical location of scallop MSTN and further QTL analysis concerning muscle mass and development will be helpful to the gene function analysis.

Quantitative analysis of C. farreri MSTN expression in embryos/larvae revealed significant differences between the stages examined, suggesting a significant role for MSTN in scallop development. MSTN has been believed to perform a key role in controlling muscle mass during mammals and fish development. (McPherron et al., 1997; Vianello et al., 2003). In mollusca, muscle was reported to first appear at the early trochophore stage and well-organized musculature is formed at veliger stage (Wanninger et al., 1999; Wanninger and Haszprunar, 2002a, b; Odintsova et al., 2006). During C. farreri development, MSTN mRNA abundance peaked at gastrula, a stage just before the early trochophore when muscle development starting, then declines at D-shaped larva and reached a minimum in veliger when musculature formation finished. The correlation between MSTN expression level and muscle development process in scallop larvae suggests that MSTN might be involved in early muscle development regulation of scallop.

In adult C. farreri, the highest expression level of MSTN was detected in striated muscle, in keeping with results for mammalian MSTNs, suggesting a conservation of function in muscle growth regulation. In addition, C. farreri MSTN was also expressed in other tissues (gonad, kidney, digestive gland, gill and smooth muscle). Similar results were obtained in bay scallop (Kim et al., 2001), and ubiquitous MSTN expression was found in fish, too (Maccatrozzo et al., 2001, 2002; Roberts and Goetz, 2003; Radaelli et al., 2003; Xue et al., 2006; Ye et al., 2007; De Santis et al., 2008). These data imply that the function of MSTN is not only restricted to muscle growth and development, but also in other developmental and physiological process. The differential tissue expression levels also suggests that the precise role of MSTN may vary between tissues. Further in-depth studies focusing on the expression regulation are required to understand the expression mechanism, and therefore, will help unveil the biological functions of MSTN in scallops.

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REFERENCES


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