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Characterization and Expression Pattern of Myostatin in the Rockfish, *Sebastes schlegeli*

Sang Beum Lee, Yong Soo Kim[1] and Hyung-Joo Jin*

Division of Marine Molecular Biotechnology, Faculty of Marine Bioscience and Technology, Kangnung National University, Gangneung 210-702, Korea

[1]Department of Human Nutrition, Food and Animal Sciences, University of Hawaii, 1955 East-West Rd., Honolulu, HI 96822, USA

Myostatin (MSTN; also known as GDF8) is a member of the transforming growth factor β-superfamily of proteins. MSTN negatively regulates mammalian skeletal muscle growth and development by inhibiting myoblast proliferation. Mice and cattle possessing mutant MSTN alleles display a 'double muscling' phenotype characterized by extreme skeletal muscle hypertrophy and/or hyperplasia. We isolated the full-length cDNA of a novel MSTN gene from *S. schlegeli* muscle tissue and examined its expression pattern in various tissues. The full-length gene (GenBank DQ423474) consists of 1941 bp with an open reading frame of 1134 bp, encoding 377 amino acids that show 62-92% amino acid similarity to other vertebrate MSTNs. The predicted protein contains a conserved proteolytic cleavage site (RXRR) and nine conserved cysteine residues at the C terminus. RT-PCR revealed that the unprocessed and prodomain myostatin mRNAs were predominantly present in muscle, with limited expression in other tissues. However, the mature myostatin mRNA was highly expressed in brain and muscle, intermediately expressed in the gills, intestine, heart, and kidney, and weakly expressed in the liver and spleen

Key words: GDF-8, Myostatin, Rockfish, *Sebastes schlegeli*, TGF-β

Introduction

The transforming growth factor β (TGF-β) superfamily includes proteins that act as negative regulators of skeletal muscle growth. One member of this superfamily, originally identified as growth and differentiation factor-8 (GDF-8), was first characterized in mice when it was discovered that disrupting the GDF-8 gene resulted in two- to three-fold increase in skeletal muscle mass (McPherron et al., 1997). This protein was later renamed myostatin (MSTN) based on the phenotype of GDF-8 null mice and the predominant expression of GDF-8 in muscle (McPherron et al., 1997). Naturally occurring MSTN mutations have been shown to cause the 'double muscling' phenotype in some breeds of cattle (Grobet et al., 1997; Kambadur et al., 1997; McPherron and Lee, 1997).

MSTN genes have been identified and isolated from a wide variety of terrestrial vertebrates, including humans (Gonzalez-Cadavid et al., 1998), mice (McPherron and Lee, 1997), cattle (McPherron and Lee, 1997), and chicken (McPherron and Lee, 1997; Kocamis et al., 1999), as well as several fish species, including zebrafish (*Danio rerio*; Xu et al. 2004; Amali et al., 2004; Biga et al., 2005), Atlantic salmon (*Salmo salar*; Ostbye et al., 2001), rainbow trout (*Oncorhynchus mykiss*; Rescan et al., 2001), brook trout (*Salvelinus fontinalis*; Roberts and Goetz, 2001), striped bass (*Morone saxatilis*; Rodgers and Weber, 2001), tilapia (*Oreochromis mossambicus*; Rodgers et al., 2001), gilthead seabream (*Sparus aurata*; Maccatrozzo et al., 2001a,b), channel catfish (*Ictalurus punctatus*; Kocabas et al., 2002a), and European seabass (*Dicentrarchus labrax*; Terova et al., 2005). In mammals the MSTN gene is expressed primarily in cells of myogenic linage, but fish exhibit MSTN expression in a variety of tissues, including muscle, gill, eyes, tongue, spleen, heart, stomach, intestine, kidney, liver, ovaries, brain, and testes (Maccatrozzo et al., 2001a, 2001b; Ostbye et al.,

*Corresponding author: hj-jin@kangnung.ac.kr

2001; Rescan, 2001; Rescan et al., 2001; Kocabas et al., 2002b; Radaelli et al., 2003; Gregory et al., 2004; Terova et al., 2005).

Previous studies have demonstrated that when MSTN expression is inactivated, muscle mass increases and fat content is reduced but all other tissues remain normal (McPherron et al., 2002; Lin et al., 2002). Conversely, MSTN overexpression has been shown to result in reduced muscle mass and increased fat content. These properties are of interest to the cattle, poultry, swine, and fish production industries given the potential for increasing meat production by inhibiting MSTN activity (Kocamis et al., 2002). The role of myostatin in muscle growth and its potential application in the aquaculture industry have prompted the sequencing of MSTN cDNAs from numerous fish species. We sequenced an MSTN cDNA and examined its expression patterns in *S. schlegeli*, one of the most important fish species in the coastal fisheries of Korea.

Materials and Methods

Sample collection

An adult *S. schlegeli* specimen was obtained from the East Sea Fisheries Research Institute (Gangneung, Korea). Various tissues, including the muscle, brain, heart, spleen, liver, kidney, intestine, and gills, were dissected and stored at -80°C until use.

Total RNA extraction

Total RNA was extracted from tissues using Trizol Reagent (Invitrogen, USA) following the manufacturer's instructions. The RNA quality and quantity were verified using spectrophotometry (Ultrospec 3100 pro, Amersham Biosciences). An 1-μg aliquot of total RNA from adult muscle tissue was reverse-transcribed using reverse transcriptase (Superscript II, Invitrogen), a CapfishingTM adaptor, and oligo dT-adaptor primers to obtain RACE-ready cDNA (CapfishingTM Full-length cDNA Premix Kit , Seegene, Korea). The cDNA was then used as a template for subsequent polymerase chain reactions (PCR).

Isolation of a partial MSTN gene

A 728-bp fragment was amplified using the DNA Walking SpeedUp Premix Kit (Seegene, Korea) with the primers, TMSP1 and TMNSP2 (Table 1). Based on highly conserved regions observed in an alignment of MSTN genes from several vertebrate species, the primers were designed to obtain the unknown sequence of the open reading frame region of the *S. schlegeli* MSTN gene. The first round of PCR was carried out using DW-ACP3 (provided by DNA Walking SpeedUP Premix Kit, Seegene) and TMSP1 (35 cycles of 94°C, 30 sec; 55°C, 30 sec; and 72°C, 100 sec). The resulting PCR product was used as a template for the second round of PCR using DW-ACPN and TMNSP2 and the same cycling conditions, resulting in one prominent 728-bp band, which was subsequently excised from the gel, purified, cloned, and sequenced.

Isolation of a full-length MSTN gene

5'- and 3'- rapid amplification of cDNA ends (RACE) was performed to generate the 5'- and 3'-terminal regions of MSTN using the Capfishing™

Table 1. Sequence of primers used in the cloning and RT-PCR

Primer	Direction	Sequence (5' - 3')
Primer for RT-PCR		
TMSP1	Reverse	CGG TTA AAG TAG AGC ATG TTG (1050-1070 bp)
TMNSP2	Reverse	CTT CTG CAA GTG CAT GTA CTC AC (944-966 bp)
Primer for 3' RACE PCR		
3'SPF	Forward	ACA GAG CCT GGA GAG GAA GGA CT (730-752 bp)
3'NSPF	Forward	GGC TGG GAC TGG ATT ATT GC (886-905 bp)
Primer for 5' RACE PCR		
5'SPR	Reverse	CGG AGC AAT AGT TGG CCT TGT AG (915-937 bp)
5'NSPR	Reverse	GAC GTC TAT ACT TTG CCA GGA GCT GA (605-630 bp)
Primers for two-step RT-PCR		
F1	Forward	ATG CAT CTG TCT CAT ATT GTG CT (1-23 bp)
F2	Forward	GAC GCG GGC CTG GAC TGT GAC (805-825 bp)
R1	Reverse	TCA AGA GCA TCC ACA ACG GTC C (1113-1134 bp)
R2	Reverse	TCT CCT GGC ACG CCT CGG GC (785-804 bp)
β-actin F1	Forward	AGT ACC CCA TCG AGC ACG GTA T (12-33 bp)
β-actin R1	Reverse	TAC GAC CAG AGG CAT ACA GGG A (230-251 bp)

Full-length cDNA Premix Kit (Seegene, Korea) according to the manufacturer's instructions. Based on the partial *S. schlegeli* MSTN sequence, we designed two sets of primers (5'-SPR and 5'-NSPR for 5'-RACE; 3'-SPF and 3'-NSPF for 3'-RACE; Table 1). The first 5'-RACE step was carried out using the 5'-RACE primer and 5'-SPR (35 cycles of 94°C, 40 sec; 58°C, 40 sec; and 72°C, 1 min). The resulting PCR product was used as a template for a second round of PCR using the 5'-RACE primer and 5'-NSPR with the same cycling conditions. The prominent 759-bp band was isolated, cloned, and sequenced. Similarly, the first 3'-RACE step was carried out using the 3'-RACE primer and 3'-SPR (35 cycles of 94°C, 40 sec; 58°C, 40 sec; and 72°C, 1 min). The resulting PCR product was used as a template for a second round of PCR using the 3'-RACE primer and 3'-NSPR and the same cycling conditions. One prominent band containing 927 bp was detected, and then isolated, cloned, and sequenced.

Expression analysis of the MSTN gene

Expression analysis was carried out using reverse-transcriptase polymerase chain reaction (RT-PCR) on total RNA from the brain, gill, heart, intestine, kidney, liver, muscle, and spleen of the *S. schlegeli* specimen. RNA (1 μg) from each sample was reverse-transcribed using oligo dT (16) as a primer. Three pairs of primers specific for the unprocessed (F1-R1), prodomain (F1-R2), or mature (F2-R1) versions of the cDNA were used to amplify 1134-, 800-, and 334 -bp fragments of the *S. schlegeli* MSTN cDNA, respectively (Table 1). The cycling parameters included an initial incubation at 94°C for 30 sec followed by 35 cycles of 40 sec at 94°C for denaturing, 40 sec at 40°C for annealing, and 1 min at 72°C for extension. A final extension step of 10 min at 72°C was performed to ensure complete extension of the amplified products. Aliquot of the PCR product from each tissue was subjected to electrophoresis on a 1.0% agarose gel. The *S. schlegeli* β-actin gene (GenBank accession number AY166590) was used as a positive control.

Sequence analysis and alignment

Similarity searches of the DNA sequence of the fragment and the deduced amino acid sequence were conducted using BLAST (http://www.ncbi.nlm.nih.gov/blast/). Species representatives for mammals (mouse AY204900), birds (chicken AY448007), and fish (*M. saxatilis* AF290910 and zebrafish AF540956) were chosen for the sequence alignment, and Poisson-corrected distances were estimated for all possible pairs. A phylogenetic tree was constructed using the neighbor-joining method based on the obtained distance matrix, and the node robustness was assessed using the bootstrap method with 1000 replications. All phylogenetic analyses were conducted using the PHYLIP program, and the MSTN phylogenetic tree was constructed using CLUSTAL W 1.83, BioEdit, PHYLIP, and TreeView software.

Results

Molecular characterization of the *S. schlegeli* myostatin gene

The full-length *S. schlegeli* MSTN (sMSTN) cDNA (GenBank accession number DQ423474) obtained using RACE consisted of 1941 bp with an open reading frame of 1134 bp, predicted to encode a 377-amino-acid protein (Fig. 1). The sMSTN sequence contains an MSTN RXRR cleavage site and nine conserved cysteine residues (Fig. 2). An alignment and phylogenetic analysis of the amino acid sequences of MSTNs of *S. schlegeli* and other vertebrates are shown in Figs. 2 and 3.

The deduced amino acid sequence of the unprocessed region of sMSTN exhibits similarity to the unprocessed regions of MSTNs from *Mus musculus* (62%), *Gallus gallus* (63%), *Danio rerio* (82%), and *Morone saxatilis* (92%). Likewise, the deduced amino acid sequence of the sMSTN prodomain region shows similarity to the prodomain regions in the MSTNs of *M. musculus* (51%), *G. gallus* (53%), *D. rerio* (77%), and *M. saxatilis* (89%). Finally, the predicted mature sMSTN is similar to the mature MSTNs of *M. musculus* (88%), *G. gallus* (88%), *D. rerio* (94%), and *M. saxatilis* (99%; Table 2). The multiple sequence alignment revealed that the mature region, located between the RXXR motif (residues 265-268, RARR, matching the RXXR consensus site) and the carboxy terminus, are highly conserved between the fish and mice sequences.

MSTN gene expression analysis

The expression levels of the MSTN gene in various *S. schlegeli* tissues, such as the brain, gills, liver, muscle, intestine, spleen, kidney, and heart, were analyzed using RT-PCR. Unprocessed and prodomain MSTN mRNA were predominantly found in the muscle, but its levels were low in the brain, gill, heart, intestine, kidney, liver, and spleen. The expression levels of the mature MSTN mRNA were high in the brain and muscle, intermediate in the gills, intestine, and kidney, and low in the heart, liver, and spleen (Fig. 4).

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GCTTCATGGGGATAGGGGATCAGTGTGGGACATTAATCCAAACCCAGTCCAGTCCAGTCGCGCATCAGGTCCAGCACACACTCACACAGGGATCTCTTTTGAAACCAAACTTGCACACCT  120

          M  H  L  S  H  I  V  L  V  L  S  L  L  V  A  L  G  P  V  V  L  S  D  Q  E  T  H  Q  Q  P  P  S  A  A  S  P  G   37
TAAGAGACAATGCATCTGTCTCATATTGTGCTGTATCTCAGCTTGCTGGTTGCTTTGGGTCCAGTGGTTTTGAGTGACCAAGAGACGCACCAGCAGCCGCCCTCTGCAGCCAGCCCAGGA  240

E  T  E  Q  C  A  T  C  E  V  R  Q  Q  I  K  T  M  R  L  N  A  I  K  S  Q  I  L  S  K  L  R  M  K  E  A  P  N  I  S  R   77
GAGACGGAGCAGTGCGCCACCTGCGAGGTCCGGCAGCAGATCAAAACCATGCGATTAAACGCGATCAAGTCTCAGATTTTGAGCAAACTGCGAATGAAAGAAGCTCCCAACATCAGCCGA  360

D  I  V  K  Q  L  L  P  K  A  P  P  L  Q  Q  L  L  D  Q  Y  D  V  L  G  D  D  N  K  D  V  V  M  E  E  D  D  E  H  A  T   117
GACATCGTGAAGCAGCTGCTGCCCAAAGCGCCGCCGCTGCAGCAGCTCCTCGACCAGTACGACGTGCTGGGAGATGACAACAAGGATGTGGTGATGGAGGAGGACGACGAGCACGCCACC  480

T  E  T  V  M  M  M  A  T  E  P  A  S  I  V  Q  V  A  E  E  P  K  C  C  F  F  S  F  S  P  K  F  Q  A  S  R  I  V  R  A   157
ACGGAGACGGTCATGATGATGGCCACTGAACCCGCGTCCATAGTCCAAGTGGCTGAGGAACCAAAGTGCTGCTTTTTCTCTTTTTCTCCAAAGTTTCAAGCCAGTCGAATAGTCCGGGCT  600

Q  L  W  V  H  L  R  P  A  T  E  A  T  T  V  F  L  Q  I  S  R  L  M  P  V  T  D  G  S  R  H  I  R  I  R  S  L  K  I  D   197
CAGCTCTGGGTTCATCTGCGACCGGCCACCGAGGCGACCACCGTGTTCCTGCAGATCTCCCGCCTGATGCCGGTCACAGACGGGAGCAGGCACATCCGCATCCGATCCCTGAAGATCGAC  720

V  N  A  G  L  S  S  W  Q  S  I  D  V  K  Q  V  L  T  V  W  L  R  Q  P  E  T  N  W  G  I  E  I  N  A  F  D  S  R  G  N   237
GTGAACGCCGGGCTCAGCTCCTGGCAGAGTATAGACGTCAAACAAGTGTTGACTGTGTGGCTGCGGCAGCCGGAGACCAACTGGGGCATCGAGATTAACGCCTTTGATTCCAGGGGAAAT  840

D  L  A  V  T  S  T  E  P  G  E  E  G  L  Q  P  F  M  E  V  K  V  S  E  G  P  R  R  A  R  R  D  A  G  L  D  C  D  E  N   277
GACTTGGCCGTGACCTCCACAGAGCCTGGAGAGGAAGGACTGCAACCGTTCATGGAGGTGAAGGTCTCAGAGGGCCCGAGGCGTGCCAGGAGAGACGCGGGCCTGGACTGTGACGAGAAC  960

S  P  E  S  R  C  C  R  Y  P  L  T  V  D  F  E  D  F  G  W  D  W  I  I  A  P  K  R  Y  K  A  N  Y  C  S  G  E  C  E  Y   317
TCTCCAGAGTCCCGGTGCTGCCGTTATCCGCTCACGGTGGACTTTGAAGACTTTGGCTGGGACTGGATTATTGCCCCAAAGCGCTACAAGGCCAACTATTGCTCCGGGGAGTGTGAGTAC  1080

M  H  L  Q  K  Y  P  H  T  H  L  V  N  K  A  N  P  R  G  T  A  G  P  C  C  T  P  T  K  M  S  P  I  N  M  L  Y  F  N  R   357
ATGCACTTGCAGAAGTATCCGCACACCCACCTGGTGAACAAGGCCAATCCCAGAGGGACCGCGGGCCCCTGCTGCACCCCCACCAAGATGTCGCCCATCAACATGCTCTACTTTAACCGA  1200

K  E  Q  I  I  Y  G  K  I  P  S  M  V  V  D  R  C  G  C  S  *    377
AAAGAGCAGATCATCTATGGCAAGATCCCTTCCATGGTGGTGGACCGTTGTGGATGCTCTTGAGTTGTGACGGAGACCCTGGCGAGAGGGAGGCCGGAGGGGCTGTGGCTCTATCCGGCC  1320

TCCGACTTCAGACTTTTTGACACAACCAATCCACCAGTTCCAATGCTTTCCTGCAGAACACGGAGCAATAGAACCAGAGTAGAGGCCACAAACAGCCCGACCTTCCCGCAGGGCAGCGCT  1440

TTCACAGCCACCATAGCTCTTACTTTTCTTTCCTCCGTCAACTGTGTCATTTAGCCATAGAGGCTTGAAGTCAGATGGATGCAGGAACATACACACACATGCTGGACCTTGGAGTGAATG  1560

TAGACAGAAATGATCAAAGTTATCCAATGTCTCTGTGTTCTGTGCTGTCCATTTATTACCCAACATGCCGACCGATTATACTCGTATGCCATCTACTCCACTCCACTCGTAGCCAACATA  1680

CAAGCTATTACACTTTTTGCTAAGCTATGTGTTTGTGATAATCATTTTTCAAGTTATGTTTTCAGAGTGAAACCAGGAATCCTCATGGACTTTTTGAAAGGGCTTGGAAAAACACAGCTG  1800

GAGACTAAAGTGATATTTCACACTGGTAGAACATGATTTAGTACACATAGGAGTCAATAAAAGGATGAAAACTGGCTACATGGCAAACCCTTATTAACTCCTATACATTATTACTGCCAT  1920

TTATAAAAAAAAAAAAAAAAA   1941
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Fig. 1. The nucleotide and deduced amino acid sequences of the *S. schlegeli* myostatin (sMSTN). The proteolytic processing site (RXRR) is underlined. The nucleotide sequence data of sMSTN appear in the GenBank database under the accession number DQ423474.

Table 2. Amino acid sequence identities of myostatin from various organisms*

	S. schlegeli	*M. musculus*	*G. gallus*	*D. rerio*	*M. saxatilis*
Unprocessed amino acid					
S. schlegeli	100				
M. musculus	62	100			
G. gallus	63	90	100		
D. rerio	82	66	66	100	
M. saxatilis	92	62	63	81	100
Prodomain					
S. schlegeli	100				
M. musculus	51	100			
G. gallus	53	87	100		
D. rerio	77	58	57	100	
M. saxatilis	89	52	52	75	100
Mature					
S. schlegeli	100				
M. musculus	88	100			
G. gallus	88	100	100		
D. rerio	94	88	88	100	
M. saxatilis	99	88	88	95	100

* Identities are given in relation to the unprocessed region, to the prodomain region and to the mature region of the MSTNs. Sources include: mouse (*M. musculus* accession number AY204900), chicken (*G. gallus* accession number AY448007), zebrafish (*D. rerio* accession number AF540956), striped sea bass (*M. saxatilis* accession number AF290910), and rockfish (*S. schlegeli* accession number DQ423474) MSTNs.

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S. schlegeli  ---MHLSHIVLYLSLLVALGPVVLSDQETHQQPPSAASPGETEQCATCEVRQQIKTMRLN 57
M. saxatilis  ---MHLSQIALYLSLLIALGPVVLSDQETHQQP-SATSPEDTEQCATCEVRQQIKTMRLN 56
D. rerio      ---MHFTQVLISLSVLIACGPVGYGDITAHQQP--STATEESELCSTCEFRQHSKLMRLH 55
M. musculus   MMQKLQMYVYIYLFMLIAAGPVDLNEGSEREEN-----VEKEGLCNACAWRQNTRYSRIE 55
G. gallus     -MQKLAVYVYIYLFMQIAVDPVALDGSSQPTEN-----AEKDGLCNACTWRQNTKSSRIE 54

S. schlegeli  AIKSQILSKLRMKEAPNISRDIVKQLLPKAPPLQQLLDQYDVLGDDNKDVVMEEDDEHAT 117
M. saxatilis  AIKSQILSKLRMKEAPNISRDIVKQLLPKAPPLQQLLDQYDVLGDDNRDVVMEDDDEHAI 116
D. rerio      AIKSQILSKLRLKQAPNISRDVVKQLLPKAPPLQQLLDQYDVLGDDSKDGAVEEDDEHAT 115
M. musculus   AIKIQILSKLRLETAPNISKDAIRQLLPRAPPLRELIDQYDVQRDDSSDGSLEDDDYHAT 115
G. gallus     AIKIQILSKLRLEQAPNISRDVIKQLLPKAPPLQELIDQYDVQRDDSSDGSLEDDDYHAT 114

S. schlegeli  TETVMMMATEPASIVQVAEEPKCCFFSFSPKFQASRIVRAQLWVHLRPATEATTVFLQIS 177
M. saxatilis  TETIMMMATEPESIVQVDGEPRCCFFSFTQKFQANRIVRAQLWVHLRQSDEATTVFLQIS 176
D. rerio      TETIMTMATEPDPIVQVDRKPKCCFFSFSPKIQANRIVRAQLWVHLRPAEEATTVFLQIS 175
M. musculus   TETIITMPTESDFLMQADGKPKCCFFKFSSKIQYNKVVKAQLWIYLRPVKTPTTVFVQIL 175
G. gallus     TETIITMPTESDFLVQMEGKPKCCFFKFSSKIQYNKVVKAQLWIYLRQVQKPTTVFVQIL 174

S. schlegeli  RLM-PVTDGSRHIRIRSLKIDVNAGLSSWQSIDVKQVLTVWLRQPETNWGIEINAFDSRG 236
M. saxatilis  RLM-PVTDGNRHIRIRSLKIELNAGVSSWQSIDVKQVLSVWLRQPETNWGIEINAFDSRG 235
D. rerio      RLM-PVKDGGRH-RIRSLKIDVNAGVTSWQSIDVKQVLTVWLKQPETNRGIEINAYDAKG 233
M. musculus   RLIKPMKDGTRYTGIRSLKLDMSPGTGIWQSIDVKTVLQNWLKQPESNLGIEIKALDENG 235
G. gallus     RLIKPMKDGTRYTGIRSLKLDMNPGTGIWQSIDVKTVLQNWLKQPESNLGIEIKAFDETG 234

S. schlegeli  NDLAVTSTEPGEEGLQPFMEVKVSEGPRRARRDAGLDCDENSPESRCCRYPLTVDFEDFG 296
M. saxatilis  NDLAVTSAEPGEEGLQPFMEVKISEGPRRARRDSGLDCDENSPESRCCRYPLTVDFEDFG 295
D. rerio      NDLAVTSTETGEDGLLPFMEVKISEGPKRIRRDSGLDCDENSSESRCCRYPLTVDFEDFG 293
M. musculus   HDLAVTFPGPGEDGLNPFLEVKVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFG 295
G. gallus     RDLAVTFPGPGEDGLNPFLEVRVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFG 294
                                          ----     *        **

S. schlegeli  WDWIIAPKRYKANYCSGECEYMHLQKYPHTHLVNKANPRGTAGPCCTPTKMSPINMLYFN 356
M. saxatilis  WDWIIAPKRYKANYCSGECEYMHLQKYPHTHLVNKANPRGTAGPCCTPTKMSPINMLYFN 355
D. rerio      WDWIIAPKRYKANYCSGECDYMYLQKYPHTHLVNKASPRGTAGPCCTPTKMSPINMLYFN 353
M. musculus   WDWIIAPKRYKANYCSGECEFVFLQKYPHTHLVHQANPRGSAGPCCTPTKMSPINMLYFN 355
G. gallus     WDWIIAPKRYKANYCSGECEFVFLQKYPHTHLVHQANPRGSAGPCCTPTKMSPINMLYFN 354
                            *   *                         **

S. schlegeli  RKEQIIYGKIPSMVVDRCGCS 377
M. saxatilis  RKEQIIYGKIPSMVVDRCGCS 376
D. rerio      GKEQIIYGKIPSMVVDRCGCS 374
M. musculus   GKEQIIYGKIPAMVVDRCGCS 376
G. gallus     GKEQIIYGKIPAMVVDRCGCS 375
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Fig. 2. Amino acid alignment of mouse (*M. musculus* accession number AY204900), chicken (*G. gallus* accession number AY448007), zebrafish (*D. rerio* accession number AF540956), striped sea bass (*M. saxatilis* accession number AF290910), and rockfish (*S. schlegeli* accession number DQ423474) MSTNs. The proteolytiic processing site (RXRR) is indicated with gray shading underlined. Conserved cysteine residues are denoted with asterisks.

Discussion

We isolated and characterized a full-length cDNA of an unknown MSTN gene in the rockfish, *S. schlegeli.* The *S. schlegeli* MSTN has common structural features with other vertebrate MSTN genes. The putative amino acid sequence contains a highly conserved carboxy-terminal region, corresponding to the mature processed protein, a potential proteolytic processing site (RARR, matching the RXXR consen-sus site) and also nine cysteine residues are present, in accordance with all known vertebrate MSTN genes (Grobet et al. 1997; McPherron and Lee, 1997; Gu et al., 2004).

Although the sMSTN gene shows high structural similarity with its mammalian counterparts, the expression pattern of MSTN genes in fish is notably different from that in mammals. In mice, MSTN is strongly expressed in skeletal muscle and weakly

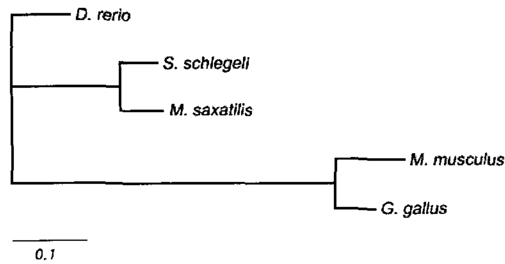

Fig. 3. Neighbor-joining phylogenetic tree of MSTN amino acid sequences of *S. schlegeli, D. rerio, M. saxatilis, M. musculus,* and *G. gallus*, based on Poisson-corrected protein distances. Phylogenetic tree of MSTNs obtained using Phylip software via the neighbor-joining method. The tree was generated via CLUSTAL W 1.83 and depicted visually via Tree-View 1.6.6. Positions containing gaps were excluded from the analysis. Numbers at tree nodes refer to bootstrap values after 1000 replicates. The scale bar refers to a phylogenetic distance of 0.1 amino acid substitutions per site.

expressed in cardiomyocytes, mammary glands, and adipose tissue (McPherron et al., 1997; Ji et al., 1998; Sharma et al., 1999). In contrast, MSTN expression was detected in several tissues of tilapia, including skeletal muscle, eyes, gill filaments, brain, gut, and gonads, but not in the liver, kidney, stomach, or heart (Rodgers et al., 2001). We found that the unprocessed and prodomain sMSTN regions were most abundant in muscle tissue, with limited expression in other tissues, whereas the mature sMSTN region is present at high levels in brain, muscle, and spleen, and intermediately expressed in the gills, liver, intestine, kidney, and heart. These data suggest that the biological actions of MSTN in lower vertebrates are not restricted to the negative growth regulation of skeletal muscle, but may also help to regulate the growth and activity of supplemental tissues. Therefore, further research is needed to understand the physiological and molecular mechanisms of MSTN in fish before it can be used to manipulate fish tissue-

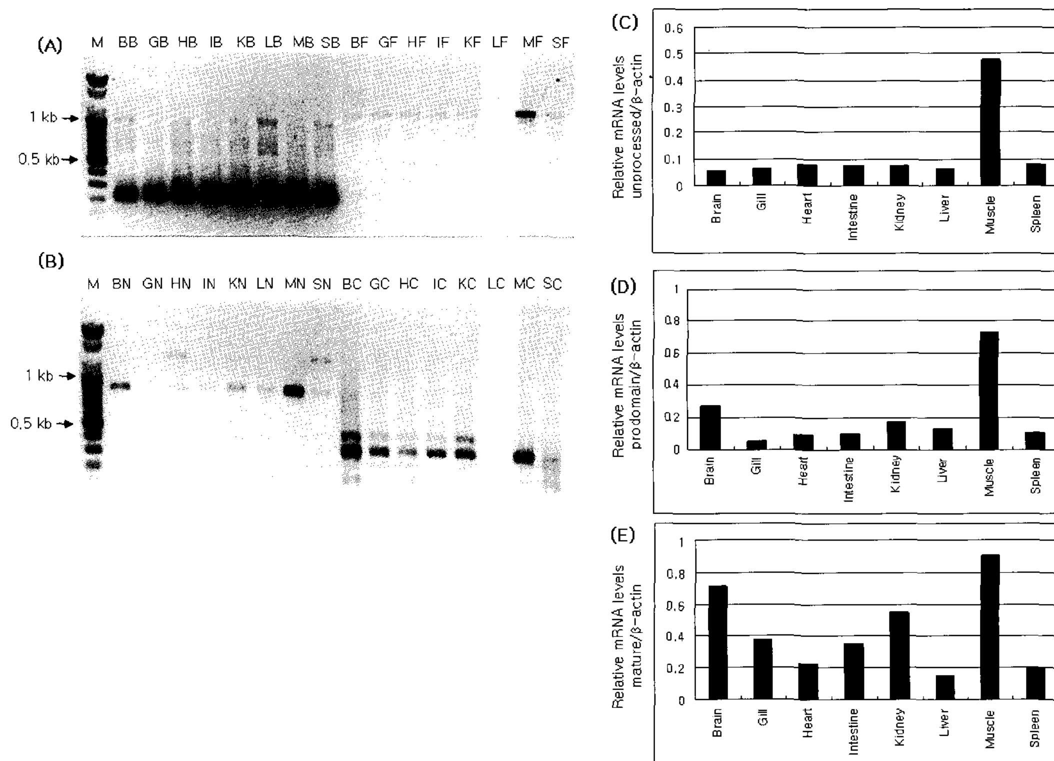

Fig. 4. Expression pattern of MSTN mRNA in various tissues of adult *S. schlegli*. One microliter of first strand cDNA was used as the template for PCR amplification of MSTN (panel A, unprocessed region: BF-SF; panel B, prodomain region: BN-SN; panel B, mature region: BC-SC) and a β-actin cDNAs fragments (panel A: BB-SB). The PCR products were fractionated on 1.0% agarose gels in TAE buffer. M, 100 bp ladder marker; B, brain; G, gill; H, heart; I, intestine; K, kidney; L, liver; M, muscle; S, spleen. The relative MSTN mRNA levels normalized by GAPDH and expressed as arbitrary units (panel C, unprocessed region; panel D, prodomain region; panel E, mature region).

mass production in the aquaculture industry.

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