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Cloning and Expression Analysis of vasa During the Reproductive Cycle of Korean Rockfish, *Sebastes schlegeli*

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Abstract Vasa, which is a conserved member of the DEAD-box protein family, plays an indispensable role in primordial germ cell proliferation. However, the expression of *vasa* gene during the reproductive cycle in ovoviviparous fish has not been documented. In this study, the full-length sequence of vasa was obtained from the ovary of Korean rockfish (*Sebastes schlegeli*) using reverse transcription-PCR and rapid amplification of cDNA ends. The Vasa with a mature protein of 650 amino acids showed greatest homology (84%) with giant gourami (*Osphronemus goramy*) and Pacific bluefin tuna (*Thunnus orientalis*). The expression of *vasa* mRNA in Korean rockfish was detected in gonads only, suggesting its specific role in gonadal development. In addition, seasonal changes in the *vasa* expression levels were examined in gonads by quantitative real-time PCR. The *vasa* transcript levels in adult testis were found higher during spermatogenesis than during spermiation. The *vasa* transcript levels remained relatively high at the early ovary stage but declined during ovary maturation in adult female fish. These results suggest that the *vasa* gene play an important role in spermatogenesis and early oogenesis during the reproductive cycle of Korean rockfish.

Key words Korean rockfish; *vasa* gene; molecular cloning; gene expression

1 Introduction

Germ cells are highly specialized to undergo meiosis and play an important role in maintaining species-specific genomic information through generations (Li *et al.*, 2010). They are derived from primordial germ cells (PGCs), which are segregated from the somatic cell lineage during early embryonic stages (Saffman and Lasko, 1999) and migrate to the developing gonad to form germline stem cells (Lin, 1997). The molecular characteristics of germ cell determinants have been studied in *Drosophila* (Rongo *et al*., 1995), and several molecular components were identified, *e.g.*, *oskar*, *vasa*, *nanos* and *tudor*. Of these, the best-characterized *vasa* gene first identified in *Drosophila* is responsible for PGCs formation and oocyte differentiation (Schüpbach and Wieschaus, 1986; Hay *et al*., 1988). The *vasa* gene encodes a putative ATP-dependent RNA helicase of the DEAD-box (asparagines (D), glutamine (E), alamine (A), and asparagines (D)) family (Hay *et al*., 1988; Lasko and Ashburner, 1988; Liang *et al*., 1994). The DEAD-box proteins, presented in a wide range of organisms and shared in 8 conserved amino acid motifs, are commonly involved in RNA metabolism such as RNA splicing, editing, rRNA processing, translation initiation, nuclear mRNA export, and RNA degradation (Lüking *et al*., 1998; Rocak and Linder, 2004).

Sequence analysis of *vasa* in diverse animal species indicates that the *vasa* gene sequence is highly conserved across animal phyla, including zebrafish (Olsen *et al*., 1997), planarian (Shibata *et al*., 1999), trout (Yoshizaki *et al*., 2000), tilapia (Kobayashi *et al*., 2000), medaka (Shinomiya *et al*., 2000), gibel carp (Xu *et al*., 2005), hydra (Mochizuki *et al*., 2000) and mouse (Fujiwara *et al*., 1994). As its mRNA is only expressed in the PGCs or germ cells in fish and several other species such as honeybee and sea urchin (Dearden, 2006; Voronina *et al*., 2008), the *vasa* gene has been used as a molecular marker of PGCs (Braat *et al*, 1999).

Research of *vasa* mRNA expression was first reported in zebrafish, *Danio rerio* (Yoon *et al*, 1997; Olsen *et al*., 1997), and then in tilapia, *Oreochromis niloticus* (Kobayashi *et al*., 2000), and gilthead bream, *Sparus aurata* (Cardinali *et al*., 2004). It has been shown that the expression of *vasa* occurs at each stage of oogenesis in fe-

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male tilapia, and at early stages rather than late stages of testis development in male tilapia (Kobayashi *et al*., 2000). The *vasa* is only expressed at the primary spermatocytes stage in Pacific bluefin tuna (Nagasawa *et al*., 2009), and its transcript levels decline from spermatogenesis to spermiation stages in catfish (Raghuveer and Senthilkumaran, 2010). However, Blázquez *et al*. (2010) have recently found that the *vasa* expression level increases during germ cell proliferation in European sea bream. In addition, Raghuveer and Senthilkumaran (2010) have indicated that *vasa* mRNA can be up-regulated by human chorionic gonadotropin in the recrudescing ovary (*in vivo*) and testicular (*in vitro*).

The Korean rockfish, *Sebastes schlegeli*, is a viviparous teleost mainly inhabiting in coastwaters of Korea, Japan, and China. It is a commercially important marine fish species with great aquacultural potential, as well as a typical ovoviviparous species for studying the evolutionary processes of reproductive control mechanism from oviparity to viviparity. In this study, the *vasa* cDNA was cloned from Korean rockfish (*Sebastes schlegeli*) to examine its distribution in tissues and temporal expression patterns during the gonadal reproductive cycle in male and female fish. Results will provide information on the reproductive physiology and endocrinology of viviparous fish and other marine fish, thus are valuable for optimization of the artificial propagation of ovoviviparous fish. In addition, the study of seasonal changes in *vasa* expression levels during the reproductive cycle will contribute to understanding of the role of vasa in spermatogenesis and oogenesis.

2 Materials and Methods

2.1 Experimental Fish

During November 2008 – September 2009, 20 mature male and female Korean rockfish were obtained every two months from the Penglai coastal area of Shandong Province, China. The gonads were excised and the sexual maturity was determined based on the presence of mature ova and sperm. Thereafter, all fish were anesthetized in tricaine methane sulfonate (MS-222, Sigma, St. Louis, MO)(Liu *et al*., 2011). Tissue samples of the brain, heart, caeca, liver, gill, head kidney, bowel, stomach, spleen, gonad and pituitary samples were collected at various growth stages, immediately frozen in liquid nitrogen and stored at −80℃ prior to total RNA extraction. Parts of the gonads were stored in Bouin's solution for hematoxylin and eosin (HE) staining so as to identify the developmental stages of gonad.

2.2 Histological Analysis

Fixed gonad segments were dehydrated in a 30%–100% ethanol series, cut into 5–8 μm-thick paraffin sections by microtome (LEICA-RM2016), stained with HE, and photographed by light microscopy (Nikon-E200, Japan).

2.3 Total RNA Extraction and Reverse Transcription-PCR (RT-PCR)

Total RNA was extracted from Korean rockfish tissues using RNAiso reagent (Takara, Japan) following the manufacturers' instructions. The concentration and purity of extracted RNA were determined by UV spectroscopy at 260 and 280 nm. Reverse transcription was carried out with RNA extracts (1 μg) and oligo $d(T)_{18}$ primers in 10 μL reactions at 70℃ for 5 min using MMLV reverse transcriptase following the manufacturer's protocol (Promega, USA).

2.4 Isolation and PCR Amplification of Vasa cDNA Fragments

For amplification of the *vasa* cDNA fragments, two pairs of degenerate primers (VasaF1/VasaR1, Table 1) were designed from highly conserved amino acid sequences of *vasa* in fish species using a web-based primer design program, Code Hop online (Rose *et al*., 1998; Chen *et al*., 2009). The PCR reaction (50 μL) contained 2 μL of cDNA from ovarian and testis tissues following the manufactures' instructions (Takara, Japan). The touchdown PCR cycling conditions were as follows: pre-denaturation at 94℃ 5 min; 10 cycles of denaturation at 94℃ for 30 s, annealing at a decreasing temperature from 70℃ to 60℃ for 30 s (decreasing 1℃ each cycle), and extension at 72℃ for 35 s, followed by additional 30 cycles of 94℃ for 30 s, 62℃ for 30 s and 72℃ for 35 s; and a final extension at 72℃ for 10 min. The PCR products were separated by a 1.5% agarose gel, purified using a TIAN gel midi Purification Kit (QIAGEN, China), cloned into pGEM-T vector (QIAGEN, China), and propagated in *E. coli* DH5α (QIAGEN, China). The clones were sequenced using an ABI3730XL sequencer (ABI, USA).

Table1 Primers and probes used for cloning, RT-PCR, and qPCR of *vasa* gene fragments

Primers	Sequence (5′–3′)	Position	Usage
VasaF1	GGCGGCTACCGGGGnaargaygarg	520–545	Degenerate primer
VasaR1	TGGGCAGCAGGAAGgcngcngtytt	918–943	Degenerate primer
Vasa-5-1	CCAACCTTTCCTCTTCCTATCATGTCCAAC	1159–1189	5′-RACE primer
Vasa-5-2	TACTCAGCCCAACCTTTCCTCTTCCTATCA	1167–1197	Nested 5′-RACE primer
Vasa-3-1	TGATAGGAAGAGGAAAGGTTGGGCTGAGTA	1168–1198	3′-RACE primer
Vasa-3-2	GAGTCAACACTGGACACCAAATACGAGACA	1087–1117	Nested 3′-RACE primer
Vasa-e-F	GGATGTTGGGTATGGGTTTTG	1231–1252	qPCR primer
Vasa-e-R	CCTCTGGATGTCCTCAGGGTA	1331–1352	qPCR primer
18SF	CCTGAGAAACGGCTACCATC	–	reference primer
18SR	CCAATTACAGGGCCTCGAAAG	–	reference primer

2.5 5′and 3′ RACE-PCR

The 5′ and 3′ RACE reactions were performed using a $SMART_{TM}$ RACE cDNA Amplification Kit (Clontech, USA) with primers listed in Table 1. The PCR reactions (10 μL) for first-strand cDNA synthesis and reverse transcription required 1 μg of total RNA and 1 μmol L^{-1} each primer. Subsequently, the full-length cDNA was cloned *via* a nested PCR using the following thermal cycling conditions: pre-denaturation at 94℃ for 5 min; 40 cycles of denaturation at 94℃ for 30 s, annealing at 69℃ for 30 s (Vasa-5-1, Vasa-3-1, Vasa-3-2) or 68℃ for 30 s (Vasa-5-2), and extension at 72℃ for 1 min; and a final extension at 72℃ for 10 min. The PCR products were run on a 1.5% agarose gel. DNA bands of the target length were excised, purified and cloned into vectors. Selected clones were sequenced on an ABI3730XL sequencer (ABI, USA).

2.6 Phylogenetic Analysis

Multiple protein sequences of *vasa* cDNA were obtained from Genbank (Altschul *et al*., 1990) and aligned with ClustalX 1.81 (Thompson *et al*., 1997). Phylogenetic analyses of full-length amino acid sequences were conducted using MEGA 4.0 (Tamura *et al*., 2007). Amino acid regions that remained unalignable were deleted using default parameters on the Gblocks serve (http://molevol.-ibmb.csic.es/Gblocks.html). Phylogenetic trees were constructed using the maximum likelihood method (1 000 bootstrapping replicates). The trees were rooted with amino acid sequences of mammals as outgroup.

2.7 Tissue Distribution Pattern of Vasa Transcripts

The expression level of *vasa* transcripts was examined in various tissues *via* RT-PCR assays. Total RNA was extracted from ovary, liver, kidney, head kidney, brain, heart, spleen, caeca, stomach, fat, gills, intestinal, pituitary of a female fish at late-vitellogenic stage and testis at spermiated stage using Trizol reagent. To avoid genomic contamination, extracted RNA was treated with RNase-free DNase I before reverse transcription. Total RNA was reversely transcribed using M-MLV RT (Promega, USA) following the manufactures' instructions, and the primers used for examination of tissue expression pattern are listed in Table 1. The tissue expression was normalized using 18S rRNA as a reference gene with the sense primer 5′-CCTGAGAAACGGCTACCATC-3′ and the antisense primer 5′-CCAATTACAGGGCCTCGAAAG-3′. PCR cycling conditions were as follows: 95℃ for 5 min; 40 cycles of 95℃ for 5 s, 58℃ for 30 s, and 72℃ for 30 s; and 72℃ for 10 min. The PCR products were checked by 1.5% agarose gel electrophoresis. The gel was pre-stained with ethidium bromide and visualized on a Gel system (Tanon, China).

2.8 Quantitative Real-Time PCR (qPCR)

The relative expression of *vasa* mRNA was determined *via* qPCR using total RNA extracted from gonads of Korean rockfish. The PCR assays were performed using Multicolor Real-Time PCR Detection System (Roche Lightcycler480, German) and iQ™ SYBR Green Supermix (Takara, Japan) according to the manufacturers' protocol. The sequences of primers targeting *vasa* (V-e-F and V-e-R) are listed in Table 1. The mRNA was treated with DNase I (Takara, Japan) and Ribonuclease Inhibitor (Takara, Japan) to remove trace genomic DNA and prevent potential genomic DNA amplification. The *vasa* qPCR conditions were as follows: 1 cycle of denaturation at 95℃ for 5 min, 40 cycles of denaturation at 95℃ for 30 s, annealing at 58℃ for 30 s, and extension at 72℃ for 30 s. As an internal control, 18S rRNA was amplified under the same conditions using Korean rockfish-specific primers (Table 1, see 2.7), and no significant changes were observed in the 18S rRNA expression level during gonadal development. The cycle threshold (Ct) values were obtained from the exponential phase of qPCR amplification, and results were analyzed using the comparative Ct method. The *vasa* expression level was normalized against 18S rRNA expression level to generate a ΔCt value (ΔCt = target gene Ct − reference gene Ct), and the relative expression of *vasa*/*18S* was analyzed according to the expression $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). The initial stage of samples was used as calibrator for comparative relative qPCR.

3 Results

3.1 Histological Characteristics of Gonads

According to previous research regarding gonadal histology of *Sebastiscus marmoratus* and *Sebastes schlegeli* (Lin and You, 2000; Lin *et al*., 2000; Yang *et al*., 2010; Shi *et al*., 2011), the testes in male Korean rockfish were classified into four categories (Fig.1). In the primary spermatogonia stage, the testes mainly had primary spermatocyte (Fig.1A); in the immature sperm stage, the primary spermatogonia divided and developed into secondary spermatogonia (Fig.1B); in the mature testes stage, germ cells in the spermatogenic cysts developed into sperms (Fig.1C); and in the post-spermiation stage, the sperms degenerated and died (Fig.1D). As for the ovary development, five stages were identified in female Korean rockfish, including the immature stage (perinucleolus stage, oocyte in the 2nd phase with clear nucleus) (Fig.1E); vitellogenic stage (primary yolk stage, oocyte in the 3rd phase with an increasing oocyte volume and decreasing nucleus volume) (Fig.1F); ovulation stage (secondary yolk stage, oocyte in the 4th phase with visible, pink- stained yolk granules) (Fig.1G) and zona radiate) (Fig.1H); and ovulation stage (tertiary yolk stage, oocyte in the 5th phase full of mature yolk granules) (Fig.1I); and gestational ovary stage (embryo formed) (Fig.1J).

3.2 Isolation and Characterization of Korean Rockfish Vasa cDNAs

The full-length *vasa* cDNA is 2 443 bp. It consists a

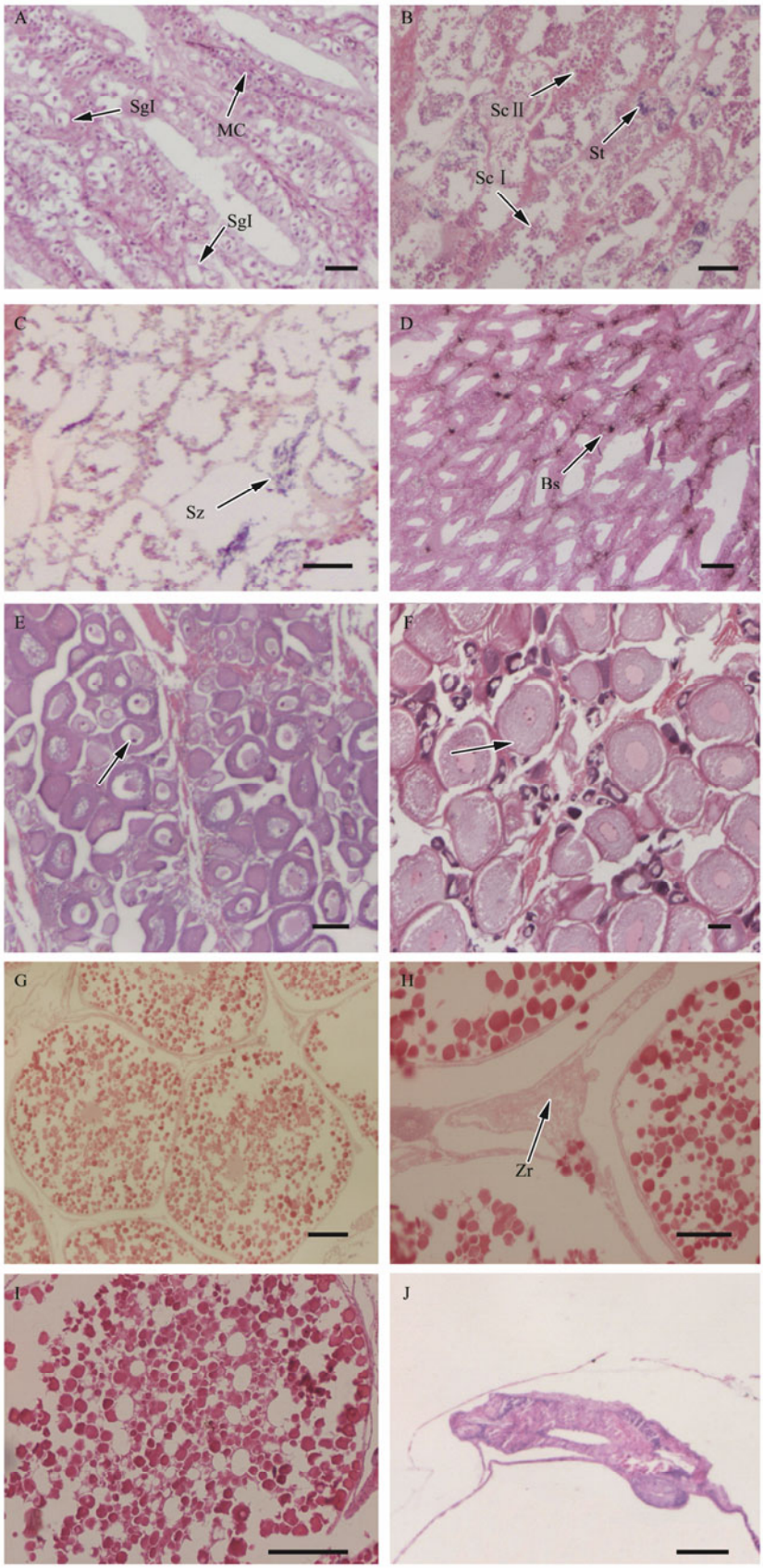

Fig.1 The histology and morphology of gonad in *Sebastes schlegeli*. A: Primary spermatogonia testis, bar = 24 μm; B: Testis filled with immature sperm, bar = 24 μm; C: Mature testis, bar = 24 μm; D: Post-spermiation testis, bar = 24 μm; E: Immature stage, perinucleolus stage oocyte at the 2nd phase, bar = 100 μm; F: Vitellogenic stage, early-oocyte at the 3rd phase, bar = 70 μm; G: Ovulation stage, post-oocyte at the 4th phase, bar = 100 μm; H: The zona radiate, bar = 100 μm; I: Ovulation stage, post-oocyte at the 5th phase, bar = 100 μm; J: Gestational ovary stage, oocyte at the 6th phase, showing the embryo, bar = 100 μm; Sg I: primary spermatogonium; ScI: primary spermatocytes; ScII: second spermatocytes; St: spermatid; Sz: spermatozoa; MC: Mesangial cell; SC: Sertoli cell; and Bs: brown substance.

1950 bp open reading frame (ORF) that encodes a 650-amino-acid protein. The predicted protein contains 8 conserved signature domains of the DEAD-box protein family, including the ATP-binding motifs (Figs.2 and 3) and the N-terminal regions with arginine-glycine (RG) and arginine-glycine-glycine (RGG) repeats. The nucleotide sequence has been deposited in Genbank (JN634874). A consensus tree was constructed with Mega 4 using the maximum-likelihood method (Hasegawa and Kishino, 1994). Phylogenetic analysis of the Vasa proteins from different fish species (Fig.4) showed the existence of 7 main clades, including mammals, birds, amphibians, teleosts, insects, mollusks and ascidians. Sequence comparison showed that the amino acid sequence of Korean rockfish Vasa had the highest homology with that of *Osphronemus goramy* (84%).

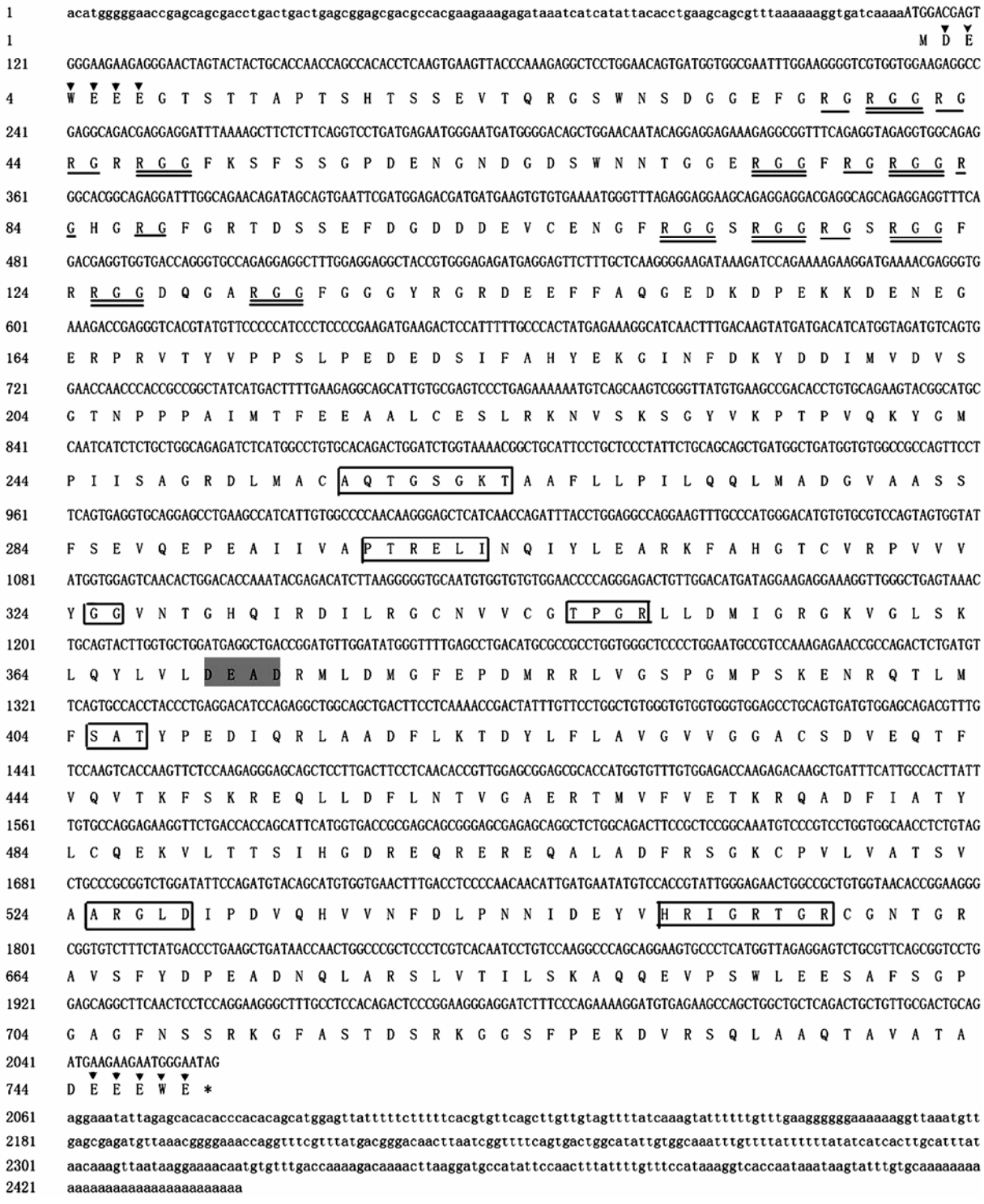

Fig.2 Nucleotide sequences and deduced amino acid sequences of Korean rockfish (*Sebastes schlegeli*) *vasa* cDNA. DEAD box shown in the gray open box; 8 conserved regions of the DEAD-box protein family shown in closed boxes; arginine-glycine (RG) repeats and arginine-glycine-glycine (RGG) repeats in the N-terminal region underlined and double underlined, respectively; acidic amino acid residues (aspartic acid, D; and glutamic acid, E) and tryptophan (W) in the N-terminal and C-terminal regions shown by triangle; asterisk (*) indicates the stop codon.

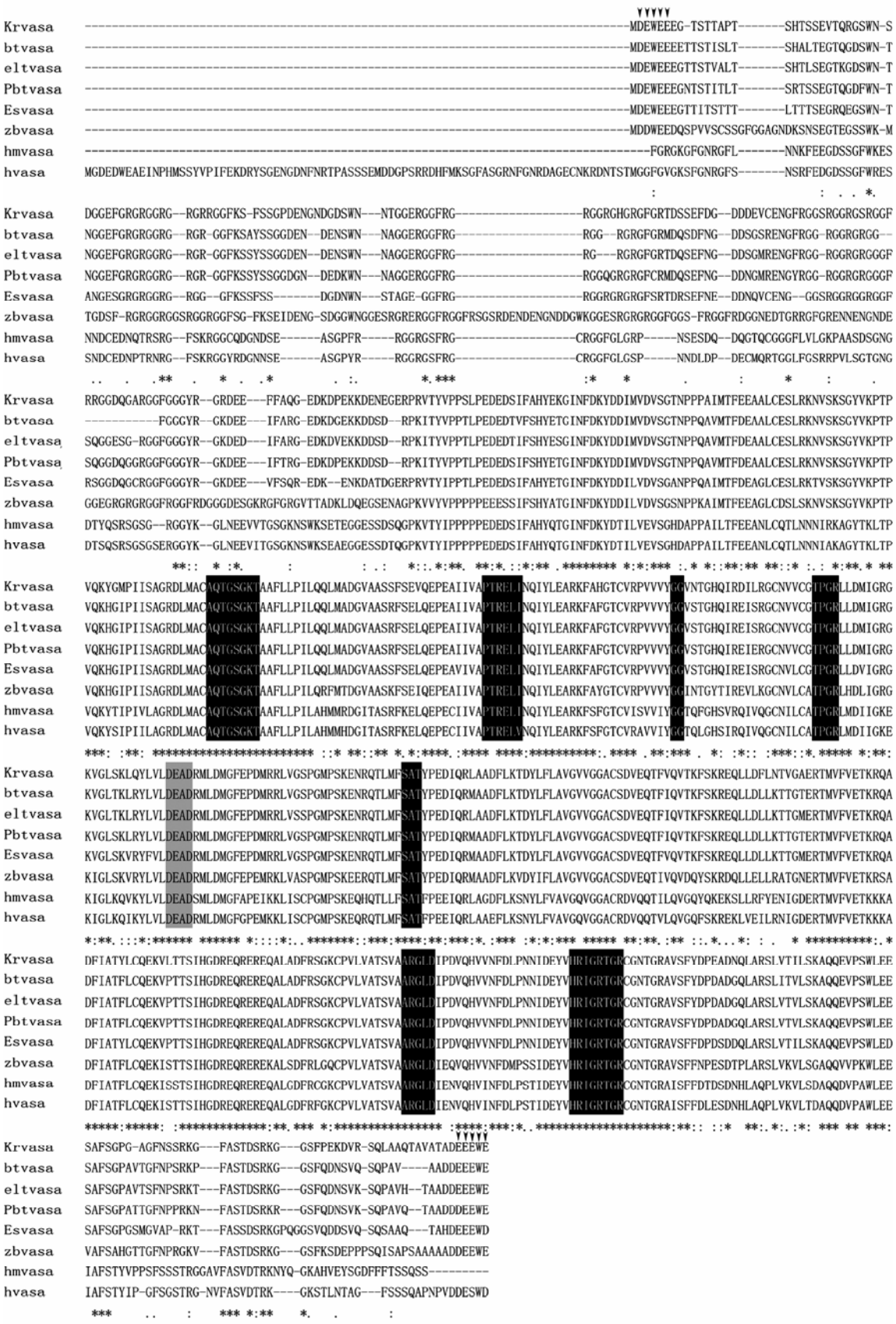

Fig.3 Amino acid sequence alignment of Vasa in Korean rockfish and other teleosts. Asterisks (*) and dots (:) marked for completely conserved and highly conserved amino acids, respectively; GenBank protein ID numbers for the sequences alignment are: *Sebastes schlegeli* vasa (Krvasa), JN634874; *Auxis rochei* vasa (btvasa), ADD81193; *Euthynnus affinis* vasa (eltvasa), ADD81191; *Thunnus orientalis* vasa (Pbtvasa), ABY77970; *Dicentrarchus labrax* vasa (Esvasa), ADK79106; *Danio rerio* vasa (zbvasa), AAL89410; *Mus musculus* vasa (hmvasa), BAA03584; *Homo sapiens* vasa (hvasa), AAF72705; acidic amino acid residues (mentioned above) in the N-terminal and C-terminal regions shown by triangle; functional (conserved) DEAD region in gray boxes; 8 conserved boxes of Vasa in black boxes.

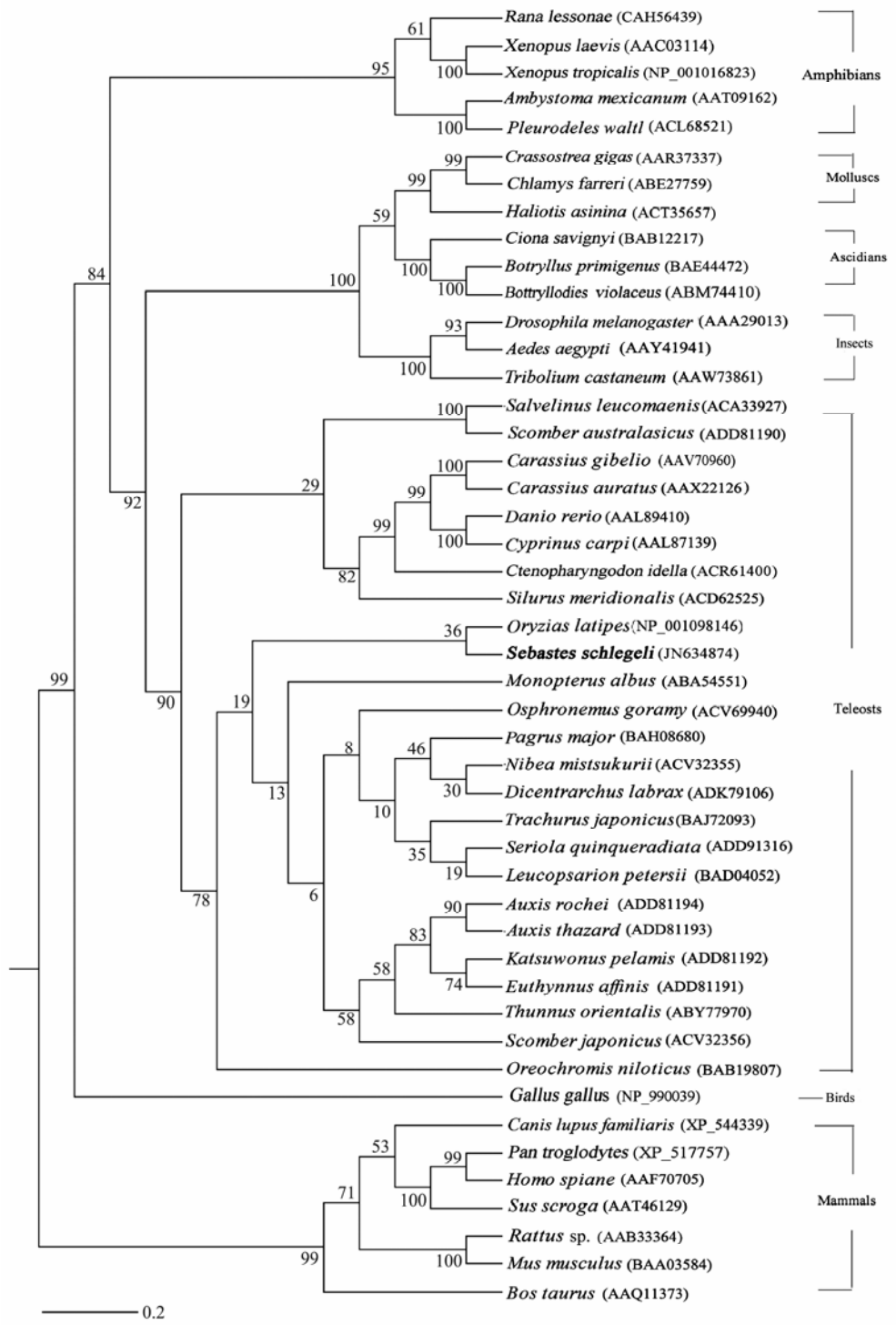

Fig.4 Phylogenetic tree based on Vasa amino acid sequences of teleost and other animal species. Accession numbers of amino acid sequences from GenBank databases shown in brackets; tree constructed using the maximum-likelihood method; bootstrap values (1 000 replicates) shown on the branches; sequences containing gaps and dubious alignments are eliminated; mammal amino acid sequences used as the out-group; branch lengths recalculated for the resulting consensus tree.

3.3 Tissue Distribution of Vasa Gene

Primers of Vasa-e-F and Vasa-e-R were used to determine specific tissue expression of *vasa*, and primers 18SF and 18SR were applied to normalize the PCR products of *vasa* mRNA and produce semi-quantitative results. RT-PCR assays revealed that the *vasa* gene was only expressed in the testis and ovary, but undetectable in other tissues (Fig.5).

3.4 Expression Pattern of vasa at Gonad Developmental Stages during the Reproductive Cycle

Expression of *vasa* mRNA was observed in gonads of both male and female Korean rockfish throughout the reproductive cycle (Fig.6). In male fish, the *vasa* expression level increased from 0.73±0.14 in the primary spermatogonia stage to 1.2±0.38 in the immature sperm stage, and then substantially decreased to 0.11±0.1 during the

mature testis stage, with the lowest value (0.02 ± 0.01) observed in the post-spermiation stage ($P < 0.05$). Different *vasa* expression levels were observed in gonads of female fish, that is, the *vasa* expression level continuously decreased from the immature stage to vitellegenic stage, and then stabilized at a low level during the ovulation stage, with the lowest level observed in the gestational stage ($P < 0.05$).

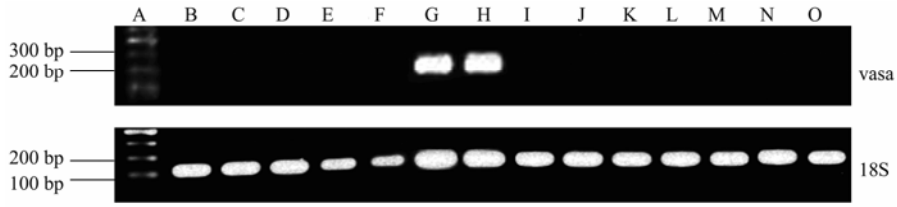

Fig.5 Expression patterns of *vasa* in different tissues of adult Korean rockfish detected by RT-PCR assay. 18S rRNA expression as internal control; A, marker; B, heart; C, liver; D, spleen; E, head kidney; F, ceaca; G, testis; H, ovary; I, stomach; J, fat; K, kidney; L, gill; M, brain; N, pituitary; and O, intestine.

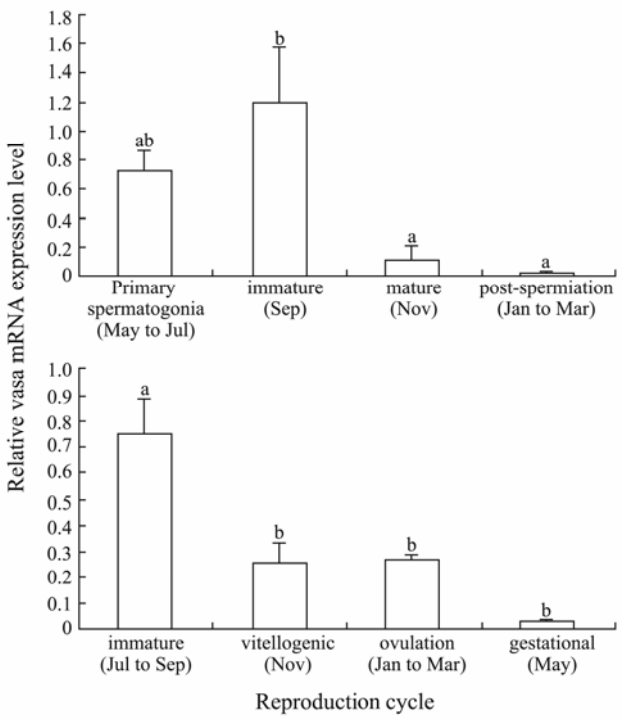

Fig.6 Relative *vasa* mRNA expression levels in gonads of male (A) and female (B) Korean rockfish during the annual reproduction cycle. Samples analyzed by qPCR; data presented as the mean normalized gene expression (MNE) levels ± standard error of the mean of triplicate samples; values normalized against the 18S rRNA expression level; different letters above the error bar showing statistical differences ($P < 0.05$) among samples collected in different periods.

4 Discussion

In the present study, the full-length *vasa* cDNA was isolated from ovarian tissue of Korean rockfish using RT-PCR and RACE amplification strategies. The Vasa protein is known to be a member of the DEAD protein family, which possesses ATP-dependent RNA helicase activity (Hay *et al*., 1988; Liang *et al*., 1994). The deduced *vasa* amino acid sequence contained 8 consensus sequences including the ATP-A (AXXXXGKT) and ATP-B motifs (DEAD) of the DEAD protein family (Linder *et al*., 1989; Pause and Sonenberg, 1992), whereas a glycine-rich region in the N-terminal region of Korean rockfish Vasa was shown to contain 7 arginine-glycine repeats and 9 arginine-glycineglycine repeats. The glycine-rich region is considered to be a characteristic of single-stranded nucleic acid-binding proteins such as RNA helicase (Hay *et al*., 1988; Kiledjian and Dreyfuss, 1992; Liang *et al*., 1994). In addition, a variety of amino acids such as glutamate (E) and aspartate (D) are acidic residues in the C-terminal region, as indicated by Fujiwara *et al*. (1994) and Castrillon *et al*. (2000). Together these demonstrate that: (1) The *vasa* gene encodes a DEAD protein with ATP-dependent RNA helicase activity; (2) The Vasa protein is highly conserved during evolution; and (3) Conserved motifs potentially play an important role in sustaining the structure and function of the Vasa protein.

In this study, we found that the Vasa protein of Korean rockfish shares high homology with those of other fish species (76% – 84%), as well as the Vasa homologs of *Osphronemus goramy* (84%). These indicate that the cDNA clone of *vasa* in Korean rockfish is a member of the Vasa family. The phylogenetic tree based on protein distances contains 7 main branches, including mammals, birds, amphibians, teleosts, insects, mollusks and ascidians (Fig.4). This suggests that the teleost Vasa protein is a single group from all other species. In addition, RT-PCR assays demonstrated that *vasa* was specifically expressed in the testis and ovary of Korean rockfish (Fig.5). Similarly, a number of reports have shown that *vasa*-related genes are specifically expressed in germline cells in different animal species, such as Drosophila (Hay *et al*., 1988; Lasko and Ashburner, 1988), Kuruma shrimp (Sellars *et al*., 2007), zebrafish (Olsen *et al*., 1997; Yoon *et al*., 1997; Krøvel and Olsen, 2004) and mouse (Fujiwara *et al*., 1994). These findings illustrate the prominent role of *vasa* in the germline development (Braat *et al*., 1999). However, it has been shown that *vasa* mRNA is weakly expressed in extra-gonadal tissues such as heart and brain in rainbow trout (Yoshizaki *et al*., 2000). Ikenishi and Tanaka (2000) suggested that *vasa* might be implicated in the translational regulation of mRNA, which is important for specification and differentiation of specific

cell types in non-gonadal tissues.

In this study, qPCR assays demonstrated different expression patterns of *vasa* mRNA in gonads of adult male and female Korean rockfish during the reproductive cycle. The *vasa* mRNA levels were found higher during the spermatogonia and immature sperm stages compared with the mature testes and post-spermiation stages in male fish. A similar decreasing expression pattern has been reported in tilapia (Kobayashi *et al*., 2000) and rainbow trout (Yoshizaki *et al*., 2000). A study of tilapia by *in situ* hybridization showed that the *vasa* signal was strong during spermatogonia and weakened with testis development (Kobayashi *et al*., 2000). In medaka, the hybridization signals have been found stronger in spermatogonia and spermatocytes at early stages, but substantially weaker in spermatocytes at later stages with no signals in spermatids (Shinomiya *et al*., 2000). These demonstrate that the *vasa* gene potentially plays an important role in the division and development of early germ cells, with a relatively minor role played in the development and maturation of late germ cells.

In female catfish, the *vasa* mRNA level has been found highest in the early ovary stage, and drastically declined with gonadal development (Raghuveer and Senthilkumaran, 2010). Similarly, our study showed that the level in female Korean rockfish decreased from the perinucleolus oocyte stage. A similar phenomenon has been observed in tilapia and gibel carp by *in situ* hybridization, and one explanation is that the amount of maternally inherited *vasa* transcript is diluted during the individual growth (Kobayashi *et al*., 2000; Xu *et al*., 2005). These findings indicate that *vasa* plays a key role in gametogensis.

In summary, the present study obtained the full-length sequence of *vasa* gene in Korean rockfish (*Sebastes schlegeli*). Spatial expression analysis substantiates that *vasa* is only expressed in gonads, with transcripts in non-gonadal tissues under the minimum detection level, including stomach, intestine, gill, and spleen. In addition, we compared the *vasa* mRNA expression at each stage of gonadal development, and the *vasa* transcript was detected at high levels at the spermatogonia and immature sperm stages in male fish and the perinucleolus stage in female fish. These suggest that *vasa* synthesis is predominant during the early reproductive stages in Korean rockfish. Future study regarding examination of Vasa in gonads at the protein level by immunohistochemistry will explore the function of Vasa in the whole reproductive cycle of gonadal development.

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