J. Ocean Univ. China (Oceanic and Coastal Sea Research)
DOI 10.1007/s11802-015-2550-z
ISSN 1672-5182, 2015 14 (4): 717-723
http://www.ouc.edu.cn/xbywb/
E-mail:xbywb@ouc.edu.cn

The Characteristics of *vasa* Gene from Japanese Sea Bass (*Lateolabrax japonic*as) and Its Response to the External Hormones

CHI Meili, WEN Haishen*, NI Meng, QIAN Kun, ZHANG Pei, and CHAI Senhao

Fisheries College, Ocean University of China, Qingdao 266003, *P. R. China*

(Received December 3, 2013; revised March 3, 2014; accepted April 13, 2015)

Abstract The RNA helicase Vasa is an important regulator of primordial germ cell development. Its function in mature fish, especially the hormone-related differences in maturing male fish has seldom been documented. In this study, a full length cDNA sequence of the *vasa* gene was cloned from Japanese sea bass, *Lateolabrax japonicas*, and it was named *jsb-vasa*. Homology analysis showed that *jsb-vasa* was closely related to its teleost homologs. The spatial distribution of *jsb-vasa* indicated that it was only highly expressed in testis, showing its germ cell-specific expression pattern. During the testicular development cycle, *jsb-vasa* was highly expressed during early period of spermatogenesis, and reduced when spermatogenesis advanced. In addition, the *jsb-vasa* gene expression was significantly inhibited at 6 h, 12 h and 24 h after injecting hCG (human chorionic gonadotropin) and GnRHa (Gonadotropin-releasing hormone analogue), indicating that *jsb-vasa* gene may play an important role in spermatogenesis of Japanese sea bass, and be under the regulation of external sex hormones.

Key words *vasa* gene; Japanese sea bass; hCG/GnRHa treatment; spermatogenesis

1 Introduction

Japanese sea bass (*Lateolabrax japonicas*) is one of the most important marine fish species cage cultivated in China. The gonadal development observation and other reproductive biology knowledge are seldom reported in this species. Because of the sharp decline in population size of wild mature Japanese sea bass and the short time of cage cultivation, it is always difficult to get enough mature male fish for artificial breeding. External hormones are often used to improving testicular development. Human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH) analogue-GnRHa are widely used in aquaculture as these hormones strictly correlate to gamete maturation in many fishes (Zohar *et al*., 2001). So they were chosen to administrate the testis development in this study.

Vasa is an ATP-dependent RNA helicase of the DEAD (Asp-Glu-Ala-Asp) family. It was identified in *Drosophila* sp. (Schüpbach and Wieschaus, 1986), and is one of the important maternal regulators of primordial germ cell (PGC) determination and development in a wide range of animals from insects to mammals (Liang *et al*., 1994; Ikenishi *et al*., 1998). Vasa protein also plays indirect roles in gene transcription, regulating the expression of transcription factors (Deshpande *et al*., 1999). Recently, the *vasa* gene has been cloned and sequenced in several teleost species, including zebrafish (*Danio rerio*) (Yoon *et al*., 1997), tilapia (*Oreochromis niloticus*) (Kobayashi *et al*., 2000), rainbow trout (*Oncorhynchus mykiss*) (Yoshizaki *et al*., 2000), medaka (*Oryzias latipes*) (Shinomiya *et al*., 2000) and gilthead sea bream (*Sparus aurata*) (Cardinali *et al*., 2004). In fish, though *vasa* gene expresses mainly in primordial germ cells, it also can be detected in the mature gonads (Xu *et al*., 2005; Mercedes *et al*., 2011). The *vasa* expression has been related to gametogenesis in tilapia, which suggests that *vasa* plays a role in the regulation of meiotic progression of male and female germ cells (Kobayashi *et al*., 2000). Moreover, the hormonal regulation of *vasa* expression during oogenesis in gilthead sea bream has also been studied (Cardinali *et al*., 2004). Since the role of *vasa* in mature gonad is unclear, and its possible involvement in gamete maturation needs to be elucidated, we detected the changes of *vasa* gene expression in Japanese sea bass by injecting hCG and GnRHa.

In this study, we cloned and characterized *vasa* gene from Japanese sea bass testis and studied its expression pattern during testicular development cycle and hCG/GnRHa administration. On these results, we might explore a possible correlation between *jsb-vasa* gene expression and the hormones involved in testis maturation

* Corresponding author. Tel: 0086-532-82031825
E-mail: wenhaishen@ouc.edu.cn

of Japanese sea bass.

2 Materials and Methods

2.1 Animal Treatment and Sampling

Seventy-one Japanese sea bass (body weight 671.21 ± 75.25 g and body length 37.78 ± 1.57 cm) were sampled from a commercial fish farm (Qingdao, China) in November 2011 and acclimatized in 16 pools for 3 days prior to the experiment. Fish were reared in natural sea water under controlled conditions (temperature 17.5 ± 0.7℃; dissolved oxygen > 6 mg L^{-1}; 13 h light:11 h dark cycle; salinity 29 ± 0.8), two third seawater was replaced daily. Then fish were randomly divided into three groups; the two treatment groups were intraperitoneal injected with GnRHa and hCG at 3.5 μg kg^{-1} body weight and 1000 units per kg body weight, respectively (Ningbo, China), while the control group injected physiological saline solution (PS) (Dabrowski *et al*., 1994; Zhang *et al*., 2001). Four male fish each group were anaesthetized with 0.2% MS-222 (Sigma, St. Louis, MO) (Liu *et al*., 2011) at 0 h, 6 h, 12 h, 24 h, and 48 h. After treatments, testes were quickly removed under sterile conditions, snap-frozen in liquid nitrogen, and stored at −80℃ for further analysis.

For the research of the testicular development cycle, six male Japanese sea bass were obtained every month during spawning season (September–December), acclimatized 3 days in laboratory and anesthetized with MS-222. Tissues including testis, liver, stomach, gills, heart, caecus, spleen, kidney, head kidney, intestine, brain, pituitary and muscle were removed rapidly, snap frozen in liquid nitrogen and kept at −80℃.

2.2 Testicular Development

The testes were fixed in Bouin's solution for more than 24 h, then dehydrated in a graded series of ethanol, embedded in paraffin and cut to 5 μm sections by microtome (LEICA-RM2016), followed by hematoxylin and eosin (H. E.) staining and photographing by light microscopy (Nikon-E200, Japan). The testis developmental stages were determined according to the method of other fishes (Otémé *et al*., 1996; Shi *et al*., 2011).

2.3 Total RNA Extraction and Reverse Transcription

Tissues from the Japanese sea bass were used for total RNA using RNAiso reagent (Takara, Japan) according to the manufacturer's protocol. Briefly, tissues were homogenized in RNAiso, precipitated isopropanol and washed in 75% ethanol. After DNase treatment, the concentration of total RNA were quantified by the Nucleic acid analyzer, Biodropsis BD-1000 (OSTC, China) and a 1.5% agarose gel was applied to detect their integrity. The reverse-transcription of 2 μL total RNA was carried out using M-MLV Reverse Transcription Kit (Promega, USA) and the resulting first strand cDNAs were used as templates.

2.4 Cloning the Japanese Sea Bass *vasa* Gene

For the purpose of obtaining the core fragment of *vasa* gene of the Japanese sea bass, two degenerate primers were designed (Vasa-F and Vasa-R, Table1) on the CODEHOP (Shi *et al*., 2011), according to four previously reported *vasa* sequences in teleost. PCR product was electrophoresed, purified then cloned into pGEM-T vector (Tiangen, China) followed by cloning in *E. coli* DH5α, and subsequently sequenced. Blasting against NCBI revealed that the cloned fragment shared high homology with *vasa* from other teleosts. Afterwards, RACE (Rapid Amplification of cDNA Ends) was carried out according to the manufacturer's protocol, with two pairs of new specific primers which were designed based on the sequence obtained above (Vasa-51 and 52, Vasa-31 and 32, Table 1). The following steps were operated as above. Multi-sequences with deduced amino acid sequences of *vasa* gene were gained from NCBI and aligned using Clustal W. MEGA 5.0 software package was applied to construct and analyze phylogenetic tree using the UPGMA method with 1000 bootstrap trials.

Table 1 Primers used for cloning *jsb-vasa*, gene expression and real-time PCR

Primer	Sequence (5'-3')	Tm (℃)	Usage
Vasa-F	GACCGGCATCAACTTCgayaartayga	65	fragment cloning
Vasa-R	TCTGCTCCACrtcrbtgcaggct	65	fragment cloning
Vasa-51	TCTGAACAGGGGTCGGCTTTACA	62	5'RACE
Vasa-52	TTTCTCAGGGACTCGCACA	60	5'RACE
Vasa-31	ACTATTTATTCTTGGCTGTGGGTG	61	3'RACE
Vasa-32	CGCTACTTTGTGCTGGACGAGGCTG	65	3'RACE
Vasa-EF	GGACACCAAATAAGGGAA	59	spatial expression
Vasa-ER	CCACAGCCAAGAATAAATAG	59	spatial expression
Vasa-QF	AAGGGAAATCTCAAGGG	56	*vasa* qPCR
Vasa-QR	CGTCCAGCACAAAGTAG	56	*vasa* qPCR
18S-F	GCGGTCGGCGTCCAACTTCT	59	qRT-PCR
18S-R	CGAGTGGGGTTCAGCGGGTT	59	qRT-PCR
Short-upm	CTAATACGACTCACTATAGGGC	–	5'RACE, 3'RACE

2.5 Tissue-Specific Expression of *jsb-vasa* Gene

A semi-quantitative PCR was set up and applied to measure *jsb-vasa* expression in thirteen tissues from male Japanese sea bass at stage Ⅴ. Total RNA (2 μg) of those tissues was isolated as described above. The18S rRNA

(control gene) primers (18S-F and 18S-R) and gene-specific primers (Vasa-EF and Vasa-ER) were listed in Table 1. To determine the amplification kinetics of each reaction, the cycle number were optimized at 14 and 28 cycles, respectively, Then the PCR products were electrophorised and analyzed by the software Chemiluminescent And Fluorescent Imaging System (SAGECREATION, China).

2.6 Real-Time PCR Assay

Real-time PCR was performed with the SYBR green (TAKARA, Japan) on Roche 480 light cycler System to measure the relative mRNA abundance of *jsb-vasa* in hormone control experiment and reproductive cycle. Two specific primers (Vasa-QF and Vasa-QR) and internal control gene primers (18S-F and18S-R) were list in Table 1. The SYBR green assay for every gene was optimized for primer concentration and annealing temperature to obtain apposite standard curve, the amplification efficiencies were 93% and 102% for *jsb-vasa* and 18S, respectively. Each qRT-PCR was carried out in triplicate. The thermal cycling parameters were an initial 1 cycle activation at 95℃ for 2 min, followed by 40 cycles of 95℃ for 15 s, 56℃ for 15 s, 72℃ for 15 s, and a dissociation curve was produced starting from 55℃ (+1℃/30 s) to 95℃. After the PCR program, $2^{-\Delta\Delta CT}$ method was used to analysis the expression level of *jsb-vasa*.

2.7 Statistical Analysis

Statistical analyses of data were performed using the SPSS 13.0, one-way ANOVA followed by Duncan's multiple range tests and differences were accepted as statistically significance when $P < 0.05$. Samples in testicular development cycle were relative to that of stage Ⅱ and samples from the hCG and GnRH-injected groups were expressed relative to that of the PS-injected group at the same time as fold change.

3 Results

3.1 Histological Analysis

Histological photomicrographs of Japanese sea bass during testicular development cycle were showed in Fig.1. At stage II, seminiferous lobular structure was in the process of formation, predominance of spermatogonia was found in lobules and the spermatogonia were round or oval with certain degree of synchronization (Fig.1A).

At stage III, testes were at early period of spermatogenesis, the lobular cavities started to enlarge and the desynchrony began to display (Fig.1B). At stage IV, male samples were in late period of spermatogenesis, and the lumen of the seminiferous lobules containing spermatozoa and lobular structure could be clearly distinguished in active spermatogenesis (Fig.1C). At stage V, testes were in spermiation stage, the lumens were filled with spermatozoa and the lobular structure of the testis was no longer distinguishable (Fig.1D).

In hormone administration, the testis at earlier period of stage V could be detected in the fish without any

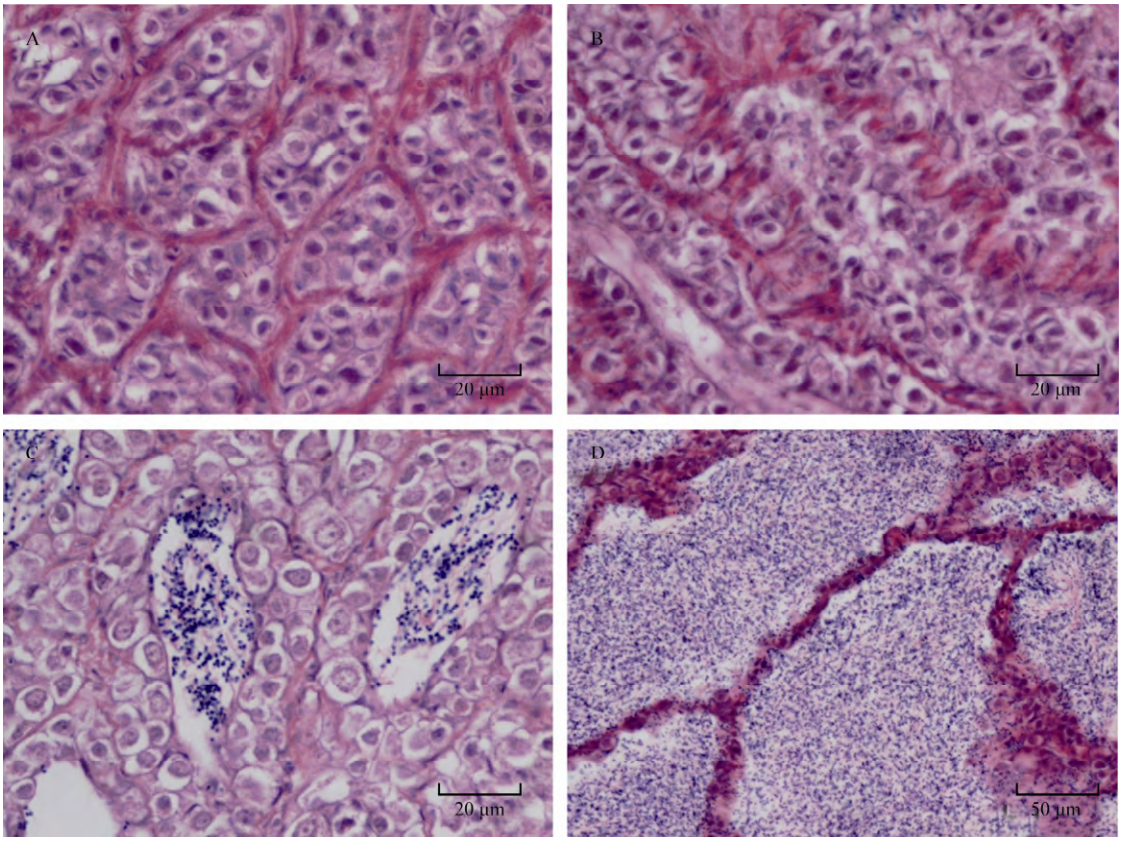

Fig.1 Histological photomicrographs of Japanese sea bass testes in testicular development cycle. (A) the testis at stage II. (B) the testis at stage III (C) the testis at stage IV. (D) the testis at stage V. Sg, spermatogonium; Sc, spermatocytes; St, spermatid; Sz, spermatozoa.

treatment. Seminiferous tubules were filled with some mature spermatozoa. After injected with the external hCG for 48 h, large quantities of mature spermatozoa were detected in the testis and the development level had been improved obviously. Moreover, the testosterone level in serum was significantly increased in hCG-treated groups at 12 h, 24 h and 48 h (data not shown).

3.2 Isolation and characterization of *jsb-vasa* cDNA sequence

The full-length cDNA of *jsb-vasa* contained an ORF of 1907 nucleotides, encoding a peptide of 632 amino acids in length (Fig.2) with a theoretical pI of 5.29 and a calculated molecular weight of 68.82 kDa. The sequence had been submitted to GenBank with an Accession No. JQ756458.1. Its amino acid sequence contained eight consensus sequences for the DEAD protein family (Fujiwara *et al*., 1994; Linder *et al*., 1989; Mercedes *et al*., 2011) including the ATPase-A (AQTGSGKT), the ATPase-B (DEAD), the RNA unwinding (SAT) and the RNA binding (HRIGRTGR) motifs (Pause and Sonenberg, 1992). Seven arginine-glycine (RG) repeats and

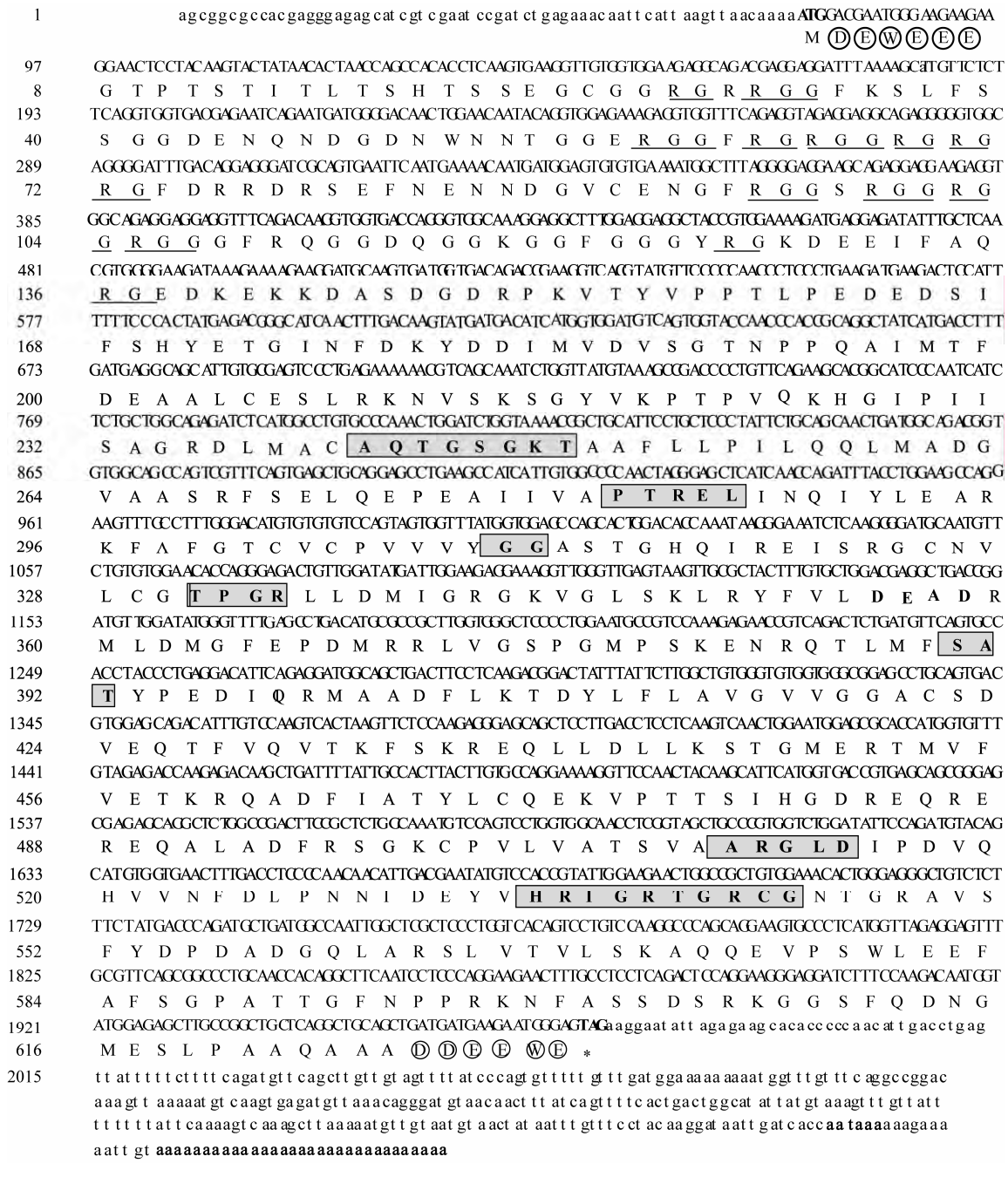

Fig.2 Nucleotide and deduced amino acid sequences of *jsb-vasa*. Eight conserved regions of the DEAD-box protein family are shaded in boxes. Glutamic acid residues (E), aspartic acid residues (D), and tryptophan residues (W) in both terminal regions are shown in circles. Seven Arginine-glycine (RG) repeats and seven arginine-glycine-glycine (RGG) repeats are underlined. The initial codon (ATG), stop codon (TAG) as well as the polyadenylation signal and the poly-A tail are marked in boldface. The GenBank Accession Number for *jsb-vasa* cDNA sequence was JQ756458.1.

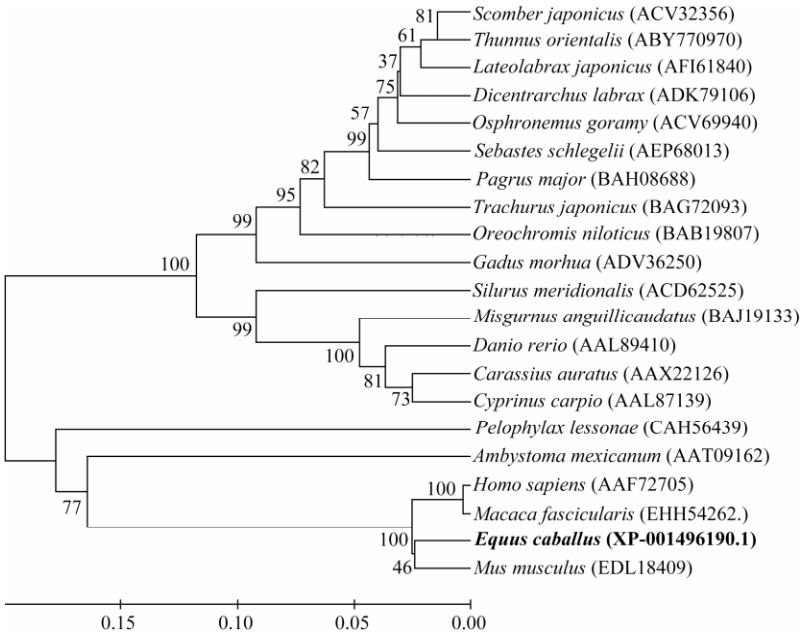

Fig.3 Phylogenetic tree of *jsb-vasa* protein. Phylogenetic analyses were conducted in MEGA version 5.0 with a bootstrap value of 1000 re-sampling. Protein sequences used for comparison and their GenBank accession numbers are listed at the right of the branches.

seven arginine-glycine-glycine triad repeats (RGG) were also found at its N-terminus. To analyze the homology of the deduced amino acid sequence with others, a phylogenetic tree was constructed, which showed three main clades including cyprinids, teleosts and mammalian and reptiles (Fig.3). Japanese sea bass belongs to the teleosts.

3.3 Tissue-Specific Expression of *jsb-vasa* in Adult Male Tissues

The expression of *vasa* gene in various tissues of adult male Japanese sea bass was analyzed by semi-quantitative PCR. Its expression was virtually restricted to the testis with a very strong signal, while no expression was detected in other extra-gonadal tissues for 28 cycles. In addition, the expression of 18S rRNA gene was found in all tissues studied at a similar intensity (Fig.4).

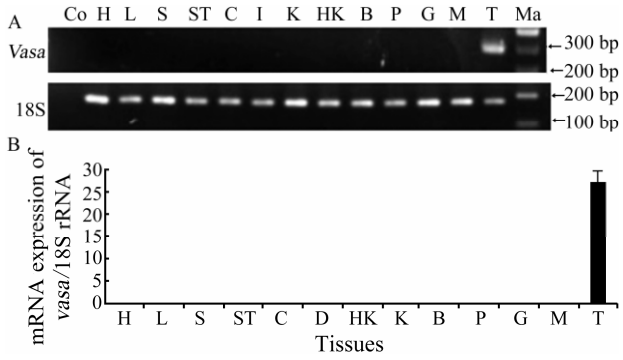

Fig.4 mRNA expression of *jsb-vasa* gene in various tissues of male Japanese sea bass at stage IV. 18S ribosomal RNA was used as an internal control for relative quantity (n=3). Control (Co, using water as template), heart (H), liver (L), spleen (S), stomach (ST), caecus (C), intestine (I), kidney (K), head kidney (HK), brain (B), pituitary (P), gill (G), muscle (M), testis (T), and DNA molecular weight marker (Ma).

3.4 *jsb-vasa* Expression in Testicular Reproductive Cycle

The variation of *jsb-vasa* expression during different stages of testicular reproductive cycle is shown in Fig.5. The relative mRNA level of *jsb-vasa* was low at stage II, increased at stage III and IV and maximized at stage IV, which was 1.4-fold higher than that at stage II. Afterward, a significant decline of *jsb-vasa* mRNA abundance was observed at stage V, which was 70% of the abundance at stage II ($P < 0.05$).

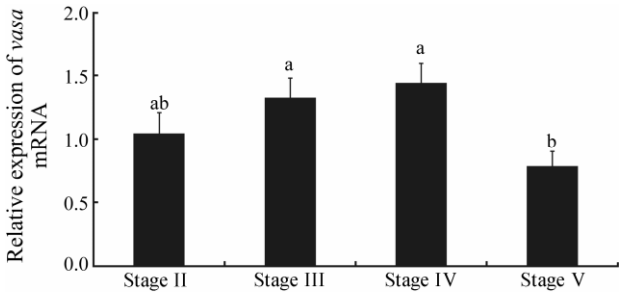

Fig.5 The mRNA expressions of *jsb-vasa* in testes during reproductive cycle. Samples were relative to that of stage II. Values are expressed as mean ± standard error. Different letters indicate significant difference ($P < 0.05$, Duncan's test).

3.5 Regulation of the Expression of *jsb-vasa* in Testes by hCG and GnRHa

Change in the relative abundance of *jsb-vasa* transcripts in response to hCG and GnRHa was assessed using real-time PCR (Fig.6). It showed that after treated

with hCG and GnRHa for 6, 12 and 24 h, the expression level of *jsb-vasa* was between 0.6- and 0.8-folds of the PS-injected control. Interestingly, treatment with GnRHa extremely decreased *vasa* mRNA abundance at 24 h, which was 0.65 fold lower than those of PS group ($P < 0.01$). In addition, the *jsb-vasa* transcript abundance remained relatively constant during the whole administration in PS-treatment group.

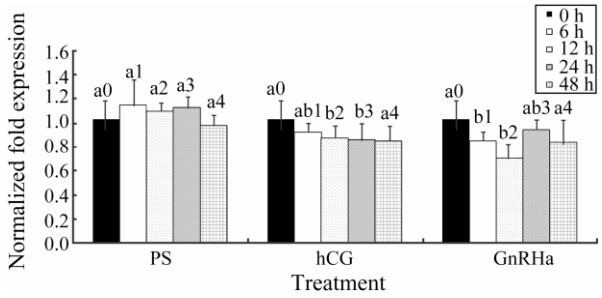

Fig.6 Regulation of the *jsb-vasa* mRNA expressions in testis by hCG and GnRHa. Data are expressed relative to control, PS-injected fish (mean ± S.E.). The *jsb-vasa* mRNA concentration had been normalized using the 18S rRNA as the internal standard by qPCR. Different letters indicate significant differences between groups at the same time after PS/hormones injection ($P < 0.05$, one-way ANOVA, followed by Duncan's test).

4 Discussion

In the present study, we isolated and characterized the full-length *vasa* gene from Japanese sea bass testis. It contained eight conserved regions of DEAD box family protein (Hay *et al*., 1988; Linder *et al*., 1989; Liang *et al*., 1994). Phylogenetic analysis revealed that Japanese sea bass vasa was more closely related to its teleost homologs. These results were consistent with the results from BLAST, exhibiting higher identity with those of European sea bass and red sea bream (91%–87% similarity) and lower similarity with common carp (*Cyprinus carpio*) and silver prussian carp (*Carassius auratus*) (82%–81% similarity) (data not shown). Together, these data suggested that the gene (cDNA) cloned from Japanese sea bass is a member of the Vasa family encoders.

Tissue distribution analysis showed that the expression of *vasa* gene was only detectable in testis. Similar expression pattern of *vasa* was also documented in other vertebrates (Kobayashi *et al*., 2000, Yoshizaki *et al*., 2000; Xu *et al*., 2005; Nagasawa *et al*., 2009). Nevertheless, expression pattern of *vasa* gene in extragonadal tissues has also been reported in many species (Ikenishi *et al*., 1998; Yoshizaki *et al*., 2000). In addition, the *jsb-vasa* mRNA abundance during testicular cycle was observed in this study. It was highly expressed in spermatogonia, a peak of expression in spermatocytes, but reduced sharply at spermatozoa stage (Figs.5 and 6). Identical or similar results were found in Korean rockfish (*Sebastes schlegeli*), zebrafish, tilapia, catfish among other teleosts (Mu *et al*., 2013; Yoshizaki *et al*., 2000; Kobayashi *et al*., 2000). However, in some mammal, like mouse and human, *vasa* expression was detected in germ cells at stages from the spermatogonium to spermatids (Toyooka *et al*., 2000; Castrillon *et al*., 2000; Kavarthapu *et al*., 2010). These results suggested that *vasa* gene might play a role in Japanese sea bass spermatogenesis.

Using hCG and GnRHa, the hormonal control of *vasa* gene expression was demonstrated for the first time by qPCR in Japanese sea bass. The *vasa* mRNA abundance was significantly inhibited after injecting hCG and GnRHa for 6, 12 and 24 h in comparison with PS group ($P < 0.05$) (Fig.6). Similar negative effect was found in gilthead sea bream (Cardinali *et al*., 2004). However, hCG treatment in catfish (*Clarias gariepinus*) testicular slices resulted in the up-regulation of *vasa* mRNA in a time-dependent manner during late pre-spawning phase (Kavarthapu *et al*., 2010). These differences may relate to the variety of species, testicular development stages, concentration of the external hormones, and the *in vivo* and *in vitro* administrations. Further studies are still needed to determine the function of *vasa* gene in regulating the transcription of other genes that are important in gonadal ontogenesis, as well as the GnRHa and hCG effects on *vasa* expression.

5 Conclusions

We cloned and characterized *vasa* gene from Japanese sea bass. Considering the change of *jsb-vasa* expression during different reproduction cycles and its response to GnRHa and hCG treatments, this gene might be under the regulation of internal and/or external sex hormones, and play an important role in spermatogenesis.

Acknowledgements

This research was supported by the National Key Technologies R & D Program of China (2011BAD13B03).

References

Cardinali, M., Gioacchini, G., and Candiani, S., 2004. Hormonal regulation of *vasa*-like messenger RNA expression in the ovary of the marine teleost *Sparus aurata*. *Biology of Reproduction*, **70** (3): 737-743.

Castrillon, D., Quade, B., Wang, T., Quigley, C., and Crum, C., 2000. The human VASA gene is specifically expressed in the germ cell lineage. *Proceedings of the National Academy of Sciences, USA*, **97** (17): 9585-9590.

Dabrowski, K., Ciereszko, A., and Ramseyer, L., 1994. Effects of hormonal treatment on induced spermiation and ovulation in the yellow perch *Perca flavescens*. *Aquaculture*, **120** (1-2): 171-180.

Deshpande, G., Calhoun, G., Yanowitz, J., and Schedl, P., 1999. Novel functions of nanos in down regulating mitosis and transcription during the development of the Drosophila germline. *Cell*, **99** (3): 271-281.

Fujiwara, Y., Komiya, T., Kawabata, H., Sato, M., Fujimoto, H., Furusawa, M., and Noce, T., 1994. Isolation of a DEAD-family protein gene that encodes a murine homolog of *Drosophila vasa* and its specific expression in germ cell lineage.

Proceedings of the National Academy of Sciences, USA, **91** (25): 12258-12262.

Hay, B., Jan, L.Y., and Jan, Y. N., 1988. A protein component of *Drosophila* polar granules is encoded by *vasa* and has extensive sequence similarity to ATP-dependent helicase. *Cell*, **55** (4): 577-587.

Ikenishi, K., 1998. Germ plasm in *Caenorhabditis elegans*, *Drosophila* and *Xenopus*. *Development Growth and Differentiation*, **40** (1): 1-10.

Kavarthapu, R., and Balasubramanian, S., 2010. Cloning and differential expression pattern of *vasa* in the developing and recrudescing gonads of catfish, *Clarias gariepinus*. *Comparative Biochemistry and Physiology, Part A*, **157** (1): 79-85.

Kobayashi, T., Kajiura-Kobayashi, H., and Nagahama, Y., 2000. Differential expression of *vasa* homologue gene in the germ cells during oogenesis and spermatogenesis in a teleost fish, tilapia, *Oreochromis niloticus*. *Mechanisms of Development*, **99** (1-2): 139-142.

Liang, L., Diehl-Jones, W., and Lasko, P. F., 1994. Localization of vasa protein to the Drosophila pole plasm is independent of its RNA-binding and helicase activities. *Development*, **120** (5): 1201-1211.

Linder, P., Lasko, P. F., Ashburner, M., Leroy, P., Nielsen, P. J., Nishi, K., Schiner, J., and Slonimski, P. P., 1989. Birth of the DEAD box. *Nature*, **337** (6203): 121-122.

Liu, M., Wen, H. S., He, F., Li, J. F., Shi, D., Hu, J., Zhang, Y. Q., Ma, R. Q., Mu, W. J., and Qi, B. X., 2011. Comp Expression patterns of cytochrome P450 aromatase genes during ovary development and their responses to temperature stress in female yellow catfish (*Pelteobagrus fulvidraco*). *Journal of Ocean University of China*, **10** (4): 409-416.

Mercedes, B., Alicia, G., Constantinos, C., and Francesc, P., 2011. Cloning and sequence analysis of a *vasa* homolog in the European sea bass (*Dicentrarchus labrax*): Tissue distribution and mRNA expression levels during early development and sex differentiation. *General and Comparative Endocrinology*, **170** (2): 322-333.

Mu, W. J., Wen, H. S., He, F., Li, J. F., Liu, M., Ma, R. Q., Zhang, Y. Q., Hu, J., and Qi, B. X., 2013. Cloning and expression analysis of vasa during the reproductive cycle of Korean rockfish, *Sebastes schlegeli*. *Journal of Ocean University of China*, **12** (1): 115-124.

Nagasawa, K., Takeuchi, Y., Miwa, M., Higuchi, K., Morita, T., Mitsuboshi, T., Miyaki, K., Kadomura, K., and Yoshizaki, G., 2009. cDNA cloning and expression analysis of a *vasa*-like gene in Pacific bluefin tuna *Thunnus orientalis*. *Fisheries Science*, **75** (1): 71-79.

Otémé, Z. J., Nunez-Rodriguez, J., Kouassi, C. K., Hem, S., and Agnese J. F., 1996. Testicular structure, spermatogenesis and sperm cryopreservation in the African clariid catfish *Heterobranchus longifilis* (Valenciennes, 1840). *Aquaculture Research*, **27** (11): 805-813.

Pause, A., and Sonenberg, N., 1992. Mutational analysis of a DEAD box RNA helicase: The mammalian translation initiation factor eIF-4A. *EMBO Journal*, **11** (7): 2643-2654.

Schüpbach, T., and Wieschaus, E., 1986. Maternal-effect mutations altering the an-terior-posterior pattern of the *Drosophila* embryo. *Roux's Archives of Developmental Biology*, **195** (5): 302-317.

Shi, D., Wen, H. S., He, F., Li, J. F., Yang, Y. P., Chen, C. F., Zhang, J. R., Chen, X. Y., Jin, G. X., Shi, B., and Li, N., 2011. The physiology functions of estrogen receptor α (ER α) in reproduction cycle of ovoviviparous black rockfish, *Sebastes schlegeli* Hilgendorf. *Steroids*, **76** (14): 1597-1608.

Shi, D., Wen, H. S., and Yang, Y. P., 2011. The annual change of ovarian development in female *Sebastes schlegel*. *Periodical of Ocean University of China*, **41** (9): 25-30 (in Chinese with English abstract).

Shinomiya, A., Tanaka, M., and Kobayashi, T., 2000. The *vasa*-like gene, ol-vas, identifies the migration path of primordial germ cells during embryonic body formation stage in the medaka, *Oryzias latipes*. *Development Growth and Differentiation*, **42** (4): 317-326.

Toyooka, T., Tsunekawa, N., Takahashi, Y., Matsui, Y., Satoh, M., and Noce, T., 2000. Expression and intracellular localization of mouse Vasa-homologue protein during germ cell development. *Mechanisms of Development*, **93** (1-2): 139-149.

Xu, H., Gui, J., and Hong, Y., 2005. Differential expression of *vasa* RNA and protein during spermatogenesis and oogenesis in the gibel carp (*Carassius auratus gibelio*), a bisexually and gynogenetically reproducing vertebrate. *Developmental Dynamics*, **233** (3): 872-882.

Yoon, C., Kawakami, K., and Hopkins, N., 1997. Zebrafish vasa homologue RNA is localized to the cleavage planes of 2-and 4-cell-stage embryos and is expressed in the primordial germ cells. *Development*, **124** (16): 3157-3165.

Yoshizaki, G., Takeuchi, Y., and Sakatani, S., 2000. Germ cell-specific expression of green fluorescent protein in transgenic rainbow trout under control of the rainbow trout *vasa*-like gene promoter. *International Journal of Developmental Biology*, **44** (3): 323-326.

Zhang, M. Z., Gao, T. X., Ruan, S. H., Zhuang, Q. Z., and Qu, Y. Q., 2001. Study on artificial cultivation and spawning inducement technique of *Lateolabrax japonicus*. *Journal of Ocean University of Qingdao*, **31** (3): 195-200 (in Chinese with English abstract).

Zohar, Y., and Mylonas, C. C., 2001. Endocrine manipulations of spawning in cultured fish: From hormones to genes. *Aquaculture*, **197** (1-4): 99-136.

(Edited by Qiu Yantao)