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Identification and Characterization of Three Differentially Expressed Ovarian Genes Associated with Ovarian Maturation in Yesso Scallop, *Patinopecten yessoensis*

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Despite great commercial interest, relatively little has been described about molecular mechanism of bivalve reproduction. We investigated genes involved in ovarian maturation of the Yesso scallop, *Patinopecten yessoensis*. GSI index and histological analysis revealed that maturation of ovary begin in February and spawning period is from April to June which is similar to the previous study in the East Sea. As result of combination analysis of differential display RT-PCR (DDRT-PCR) and histological examination, vitellogenin (Vg), ferritin (Ft) and ADT/ATP carrier protein (ACC) were identified as differently expressed genes in maturating ovary. End-point RT-PCR results showed that Vg is ovary-specific genes whereas Ft and ACC are expressed ubiquitously suggesting that Vg can be good molecular markers for ovarian development and sex determination in bivalves. Quantitative PCR results revealed that Vg were expressed highest during growth stage and appears to play a major role in oocyte maturation. On the contrary, expression of Ft was highest after spawning stage, which suggests that up-regulation may be involved in spawning and inactive stages in which the scallops recover from spawning. In addition, high level of the mitochondrial gene, ACC, may play a role in energy metabolism in maturating oocytes. Isolation and molecular studies of these key genes will expand our knowledge of the physiological changes from various exogenous factors including temperature, salinity, pH, even or numerous endocrine disrupting chemicals (EDCs) during reproductive cycle. In addition, further study of these genes implicates various industrial applications including the stable seed production, increased food quality, or economic aquaculture system.

Key words: Biomarker, Bivalve, Scallop, Vitellogenin, Maturation, *Patinopecten yessoensis*

Introduction

Understanding the reproductive mechanism is a key to develop a regular supply of seed for the aquaculture industry. However, knowledge of molecular reproduction mechanism has been fragmental due to the lack of genomic information and ambiguity of endocrine system in bivalves. For last decade, several studies provided useful information to expand our knowledge of bivalve reproduction. Gonad development in bivalves appears to be regulated by serotonin (5-hydroxytryptamine or 5-HT) and steroids. 5-HT induced oocyte maturation and spawning in clams (Fong et al., 1998; Hirai et al., 1988), which is mediated by a Ca^{2+}-dependent signaling pathway (Abdelmajid et al., 1994). Sex steroids also appear to be involved in gonadal development and sexual maturation. Administration of estradiol, testosterone and progesterone stimulated oogenesis in the scallop, *Mizuhopecten yessoensis* (Varaksina and Varaksin, 1991). Additionally, estradiol induced synthesis of vitellogenin and vitellogenin-like protein in *Patinopecten yessoensis* (Osada et al., 2004b), *Crassostrea gigas* (Li et al., 1998), and *Elliptio complanata* (Gagne et al., 2001). However, cloned estrogen receptor orthologs from mollusks did not

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bind estrogen while they were constitutively active (Kajiwara et al., 2006; Keay et al., 2006). In addition to sex steroids, gonadotropin-releasing hormone receptor (GnRHr) orthologues were also isolated from bivalve, *Crassostrea gigas* (Rodet et al., 2008) and cephalopod, *Octopus vulgaris* (Kanda et al., 2006). The results strongly support the idea that bivalve mollusks share many common characteristics in reproduction endocrine system with vertebrates.

The scallop, *Patinopecten yessoensis*, is a cold-tolerant species native to the northern Pacific Ocean. It is one of the most important shellfish resources and its demands increase annually and its production reached more than 1.2 million metric tons in 2001 (FAO, 2003). High demand has driven the growth of a scallop aquaculture industry in Asian countries, including Korea, Japan, and China. However, traditional seed production and aquaculture methods have been facing crisis from climate change and environmental contamination from various pollutants. To sustain productivity from those dramatic environmental changes in aquaculture system, profound knowledge of reproduction mechanisms is needed. Gene marker technologies can be used to widen those limited knowledge and to sustain the high production efficiency and financial profitability of aquaculture industries (Davis and Hetzel, 2000). Genes that are expressed at specific developmental stages are potential molecular markers to characterize physiological changes that occur during the reproductive cycle. Recently, genes associated with gonadal development, such as vitellogenin, gonadotropin-releasing hormone receptor, and oocyte maturation-arresting factor, have been isolated from bivalves (Osada et al., 2004b; Rodet et al., 2005; Rodet et al., 2008; Tanabe et al., 2006). In the present study, we used Differential Display RT-PCR (DDRT-PCR) technique to identify mRNA transcripts differentially expressed during oocyte maturation and development. We isolated three genes (vitellogenin, ferritin, ADP/ATP carrier protein) and measure their transcript level throughout year-round reproduction cycle to estimate roles of each gene.

Materials and Methods

Experimental animals

Live *P. yessoensis* used in this experiment were cultured in the East Sea Fisheries Research Institute from October 2007 to September 2008. Six individuals (three male and female) were collected every month. Total weight (TW), meat weight (MW), gonad+visceral weight were measured before dissection. GSI (gonad-somatic index) was calculated by the following formula: gonad+visceral weignt/ meat weight ×100. The dissected tissues were divided into two pieces. One half was used for the histological study and the other half was kept at -80°C until they used for gene expression experiment.

Differential-display RT PCR

Differential-display RT-PCR (DDRT-PCR) technique (GeneFishing™ DEG Premix Kit; Seegene Inc., Korea) was employed to isolate genes that were transcriptionally controlled during reproduction cycles. DDRT-PCR is generally accepted as one of the cost-effective techniques to isolate differently expressed genes (Kim et al., 2008; Kim et al., 2004). Three male and female individual samples were chosen from both inactive and late growth stage based on the histological examination. In order to rule out individual variations, we performed the PCR in triplicate with three different individuals. To obtain cDNAs from the gonads in different developmental stages, frozen tissues were homogenized by Polytron homogenizer (Brinkmann, Westbury, USA). Total RNA was isolated using the Trizol reagent according to the manufacturer's instructions (Invitrogen, USA). Isolated total RNAs were quantified, alliquoted, and stored at -80°C until they were used for cDNA synthesis. Before the cDNA synthesis, contaminated genomic DNA was digested by treating with DNase I (Promega, USA) at 37°C for 10 min and followed by at 70°C for 10 min. 2 μg of total RNA, 1 μL of dT-ACP 1 and dNTP (2.5 mM) were mixed together and stored at 70°C for 1 min. 5x First-strand buffer 4 μL , 0.1 M DTT 2 μL and RNase out 1 μL were added to the mixture and incubated at 42°C for 90s. Finally, 1 μL of Super script II reverse transcriptase (Invitrogen, USA) was added and incubated at 42°C for 50 min. Synthesized cDNA was quantified by spectrometry (Nanodrop Technologies, Inc.) and 100 ng of cDNA was used for each PCR reaction. The reaction mixture (30 μL) contained cDNA 3 μL,10x buffer (Takara Bio Inc., Japan) 3 μL, dNTP 2 μL, 20 pM dT-ACP2 1 μL, 20 pM arbitrary ACP 1 μL, rTaq 0.15 μL, and distilled water 20 μL. The PCR reaction was carried out with first cycle (94°C for 1 min, 50°C for 3 min, 72°C for 1 min) and followed by 40 cycle (94°C for 40s, 65°C for 40s, 72°C for 40s). PCR products were separated by 1.5% agarose gel electrophoresis and stained with ethidium bromide (EtBr). Differentially amplified bands were isolated using Gel DNA Extraction Kit (Bioneer Inc., Korea) and ligated using pGEM-T Easy Cloning Kit (Promega, USA). Ligated inserts were identified by PCR with M13forward

primer (5'-CAGGAAACAGCTATGACC-3') and M13 reverse primer (5'-TTGTAAAACGACGGCCAG-3'). Positive clones were cultured by shaking incubator (30 rpm) at 37°C for overnight. Plasmids were isolated by column-type plasmid prep kit (Bioneer, Korea) and DNA sequence was determined by automated DNA sequencer (ABI Biosystem, USA). The nucleotide sequence similarities were analyzed by BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/).

Expression analysis of three genes during oocyte maturation

Expression of each gene was determined by both end–point and quantitative RT-PCR. cDNAs from ovary, testis, gill, digestive gland, adductor muscle, mantle tissues were prepared by the method described above ,except for random hexamer instead of dT-ACP1. All the primers were designed and evaluated by IDTSciTools program (http://www.idtdna.com/SciTools/Sci Tools.aspx). Total volume of 20 μL PCR reaction contained quantified cDNA(50ng) 1 μL, 4 pM forward primer 1 μL, 4 pM reverse primer 1 μL, 10x buffer 2 μL, dNTP 2 μL, Takara ExTaq 0.1 μL, and distilled water 13 μL. Conditions were 94°C for 1min, 25cycle (94°C for 1min, 62°C for 30s, 72°C for 30s). Quantitative assay was carried out by real-time PCR using DNA Engine Opticon 2 Real Time PCR Detection System (Bio-Rad, USA). Primers used for end-point RT PCR were also used for Real- time PCR. Real time PCR mix contained 2x SYBR green remix Ex TaqII (Takara Bio Inc., Japan) 10 μL, 4 pM forward and reverse primer 2 μL, quantified cDNA (20 ng) 5 μL, distilled water 1 μL. PCR conditions were as followed ; initial reaction at 94°C for 1min followed by 40 cycle of denaturation at 94°C for 30s, annealing at 60°C for 30s and elongation at 72°C for 30s. Dissociation curves of PCR products were also obtained from 65°C to 95°C at 0.2°C interval. Standard curve using quantified copy numbers was used as reference (Kim et al., 2005). Calculated copy number was then normalized by 28s rRNA copy number. 28s rRNA primers were used to F870 (5'-CCCGTCTTGAAACACGGACCA-3') and R1200 (5'-TTCGATTAGTCTTTCGCCCCTAT-3').

Histology

All experimental animals were dissected at each stage. The gonad is located between the gill and the adductor muscle. The sex and developmental stage of each gonad was determined by microscopic examination. Dissected gonad was fixed in Bouin's solutions for 1 day and washed in running tap water. After washing, specimens were dehydrated in a series of gradient alcohols and embedded in paraffin. The paraffin blocks were trimmed and then sectioned at 5 μm with a microtome. Sections were stained with Harris's hematoxylin and eosin, and mounted on a slide using marynol (Muto Pure Chemicals Co., Japan). The developmental stage was divided into four categories: 1) growth, 2) ripe, 3) spent and 4) inactive (Dukeman et al., 2005).

Data analysis and Statistics

Multiple amino acid sequence analysis was performed using the ClustalW program (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and represented by the GeneDoc program (http://www.nrbsc.org/gfx/genedoc/index.html). All the graphs were constructed by Sigma Plot 2001. The significance of expression level in three genes was evaluated statistically by comparing the means, applying non-parametric tests followed by Mann-Whitney Test using Statistical Package for the Social Sciences (SPSS) (version 10.1). The results were considered significant at $P<0.05$.

Results

Comparison of reproduction cycle of Yesso scallop

GSI and histological observations were documented monthly for one year. Fig. 1a shows annual pattern of the GSI of *P. yessoensis* collected from the East Sea. A clear seasonal change in mean GSI was observed as shown in previous research ranging from 13.02 to 25.14 (Uddin et al., 2007). The occurrence of partially spawned gonads indicated that spawning commenced in April. Collectively, overall reproductive cycle was similar to the previous reproductive cycles.

Histological examination was also carried out in each month for one year to determine reproductive stages. Oogenesis commenced in November and oocytes grow on towards the center of the lumen and occupy about half of the lumen volume during growth stage (Fig. 1b). During ripe stage, oocytes have been fully grown and surrounded by gelatinous membrane and each cytoplasm is filled with a large number of yolk granules (Fig. 1b). Oocytes are discharged and the lumen becomes partially empty during the spawning stage (Fig. 1b). In inactive stage, observed cellular breakdown was occurred after the spawning stage and follicles are mostly empty during from July to October (Fig. 1b-I). Most gonads were shown to be in the growth stage from November to January and gonads from February and March were

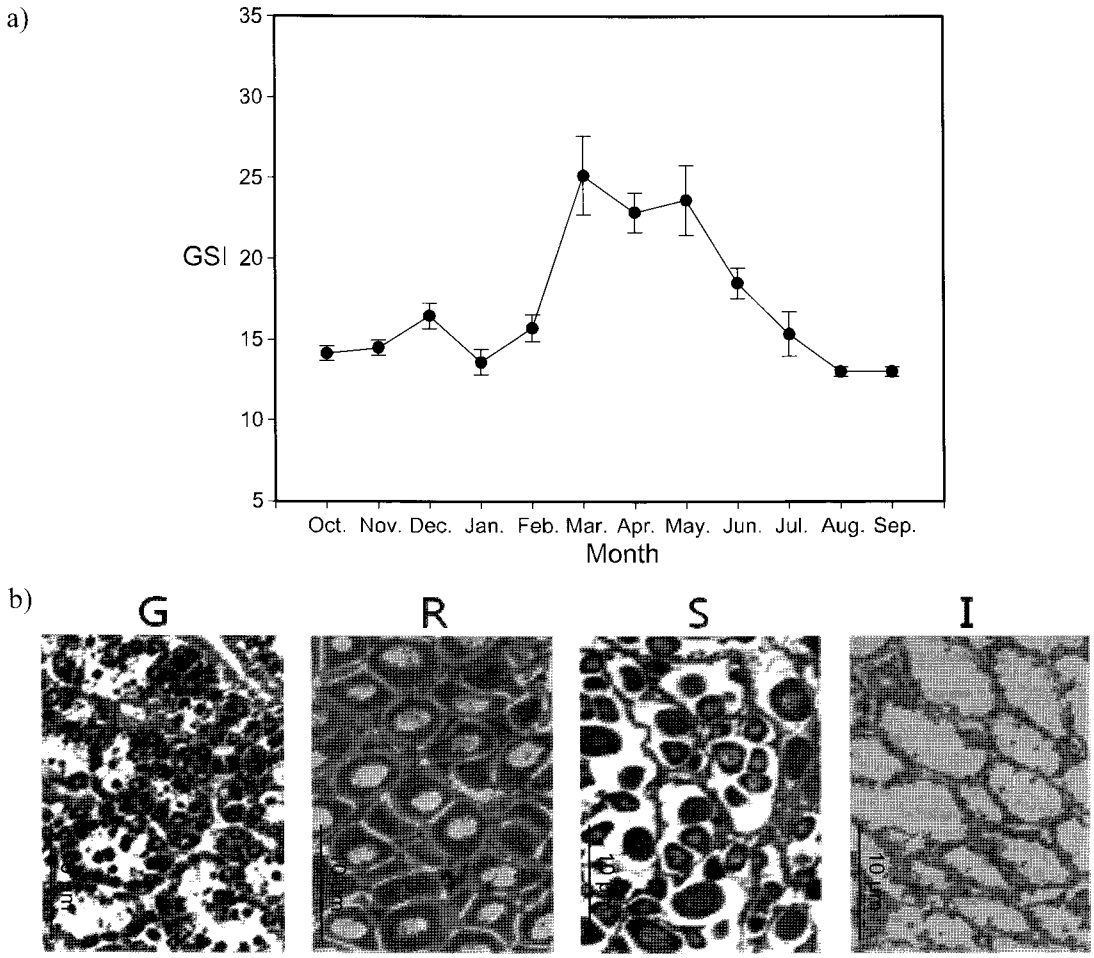

Fig. 1. Annual reproductive cycle of *P. yessoensis*. a, Seasonal variation of gonad-somatic index (GSI).Bars represent the standard error of the mean values; b, Microphotographs of the ovarian developmental phases of *P. yessoensis* (×200). G, growth stage. Oocytes grow on towards the center of lumen and occupy about half of the lumen volume and some attached in ovary; R, ripe. The ovarian tubule reaches to the largest volume in all stages and lumen is filled in ovary; S, spawning; the lumen becomes distinctly empty; I, inactive. After the spawning, cytolysis occurred and follicles mostly empty in ovary.

in the ripe stage. Gonads from April were either in the ripe or spent stages and most were in the spent stage in May and June.

Three genes specifically changed during maturation stage

Three replications of DDRT-PCR results using 120 different primers confirmed three PCR products between inactive stage and growth stage in scallop, *Patinopecten yessoensis* (Fig. 2). Primer set ACP 85 amplified genes expressed higher in growth stage whereas ACP 8 and ACP 60 produced prominent PCR bands in the inactive stage. upon cloning and determination of nucleotide sequences, PCR product of ACP 85 (784 bp) shared 98% nucleotide sequence identity with vitellogenin (Vg) from *M. yessoenesis*, in which only 4 nucleotides were different from the database. This difference may have come from the populational polymorphism among same species suggesting the gene amplified from ACP 85 primer set is Vg. Interestingly, Vg gene identified in this study did not show the stop codon and 3' UTR region. The clone with exactly the same 3' end was also shown in the previous study (Osada et al., 2004a). Since Northern blot analysis identified a single transcript longer than 10kb, Vg gene in this study may have been amplified by the landing of poly T region of the ACP85 on a series of multiple adenosine sequence within coding region. In addition to Vg which are already in the NCBI database, the PCR product from ACP8 exhibited the highest amino acid sequence identity (97%) with ferritin (Ft) from the

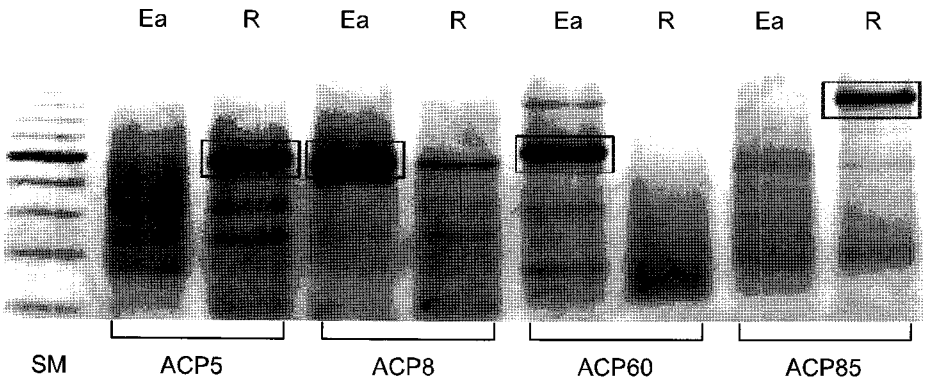

Fig. 2. Differentially expressed genes in maturating oocyte in *P. yessoensis*. PCR products from inactive (I) and growth (G) were separated on the 2% agarose gel. Three differently amplified PCR products are boxed. The number of primer set were shown at the bottom of the each lane. Marker was showed as SM.

Table 1. Oligonucleotide primers used for expression study

Name	Sequence	Description
PYODEG5RTF	5'-TTATCCCAATGGCGGCGAGAAACAAC-3'	Specific forward primer for PLRP
PYODEG5RTR	5'-AAATGGCGGACTTCCTGTGGTTGACA-3'	Specific reverse primer for PLRP
PYODEG8RTF	5'-TGGAGGAACAAGTGGAGGACATCA-3'	Specific forward primer for Ferritin
PYODEG8RTR	5'-GTTCCTTTGAACACCTCCCGTA-3'	Specific reverse primer for Ferritin
PYODEG60RTF	5'-ATGTCCTTGATGAAGGGTGCTGGA-3'	Specific forward primer for ADP/ATP carrier protein
PYODEG60RTR	5'-TCAGGTCCACTGTTCCAACATAGT-3'	Specific reverse primer for ADP/ATP carrier protein
PYODEG85RTF	5'-GAACAGCAGTGGTCATTGCCACAA-3'	Specific forward primer for Vitellogenin
PYODEG85RTR	5'-CAGCTGGCAGCATGTCAATCAGAA-3'	Specific reverse primer for Vitellogenin
28S rRNAF870	5'-CCCGTCTTGAAACACGGACCA-3'	Forward primer for 18S rDNA
28S rRNAR1200	5'-TTCGATTAGTCTTTCGCCCCTAT-3'	Reverse primer for 18S rDNA

Table 2. Genes identified by comparing amino acid sequence with NCBI data base

Primer No.	Genes	E - value	Size	Species	Gene bank No.
ACP8	Ferritin	6e-15	338 bp	*Chlamys farreri*	AAV66904
ACP60	ADP/ATP carrier protein	7e-23	445 bp	*Haliotis diversicolor*	ABY87392
ACP85	Vitellogenin	6e-139	784 bp	*Patinopecten yessoensis*	BAB63260

different scallop species, *Chlamys farreri*. Finally, PCR product obtained from ACP60 primer set product turned out to be homolog of the mitochondrial ADP/ATP carrier protein (ACC) from the abalone, *Haliotis diversicolor*. Collectively, identities of all three genes were determined by amino acid similarity analysis.

Expression of three genes

To understand expression pattern of three genes, we performed end-point RT-PCR (Fig. 3). Tissues were collected from one female in the growth stage and testis tissue was dissected from the male individual in the growth stage. After 25 cycles of amplification, PCR products of 28S rRNA were identified in all six tissues (ovary, testis, gill, digestive gland, adductor muscle, and mantle), which proved that all synthesized cDNA were good. Two genes, Ft and ACC, were expressed in all six tissues showing the ubiquitous expression profile. In contrast, Vg was expressed predominantly in ovary and low in testis. This result suggest that Vg is gonad-specific genes, especially, oocyte-specific. Quantitative PCR was carried out to understand the relationship between ovarian reproduction cycle and gene expression profile (Fig. 4). Three samples were collected from each maturation stages based on the histological examination. The relative copy numbers were normalized by the number of 28S rRNA. ACC outnumbers other genes in all stages (614.34 to 4820.11), followed by Vg, and Ft (Table 3). Expression of Ft was 10-fold higher in spawning and inactive stage than in the growth and ripe stages suggesting its involvement in spawning and inactive stage. A 7.8-fold higher expression of ACC in growth stage was observed compared with in ripe stage. This may have come from the increase in cell number and energy requirement during the growth stage. The highest number of Vg transcripts was detected during growth stage whereas only basal expression was recognized

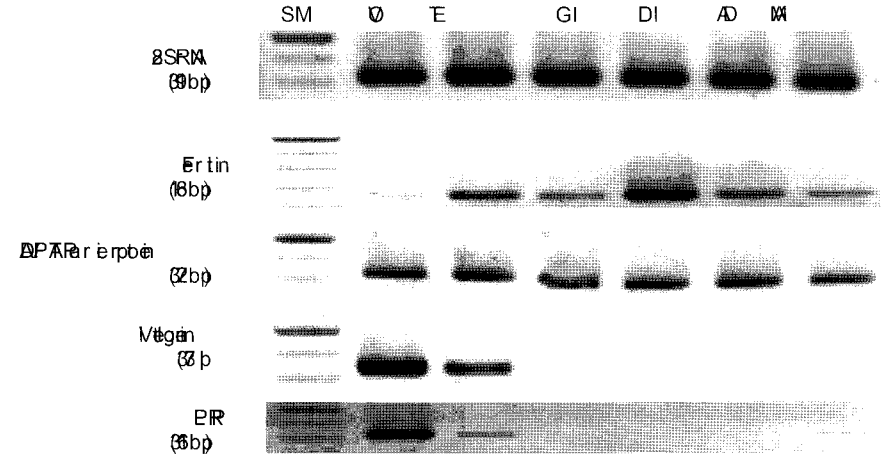

Fig. 3. Expression of three genes in various tissues. End-point RT-PCR was carried out for 25 cycles and products were separated on 1.5% agarose gel. The 28S rRNA was used as a positive control. OV, ovary; TE, Testis; GI, gill; DI, Digestive gland; AD, adductor muscle; MA, Mantle.

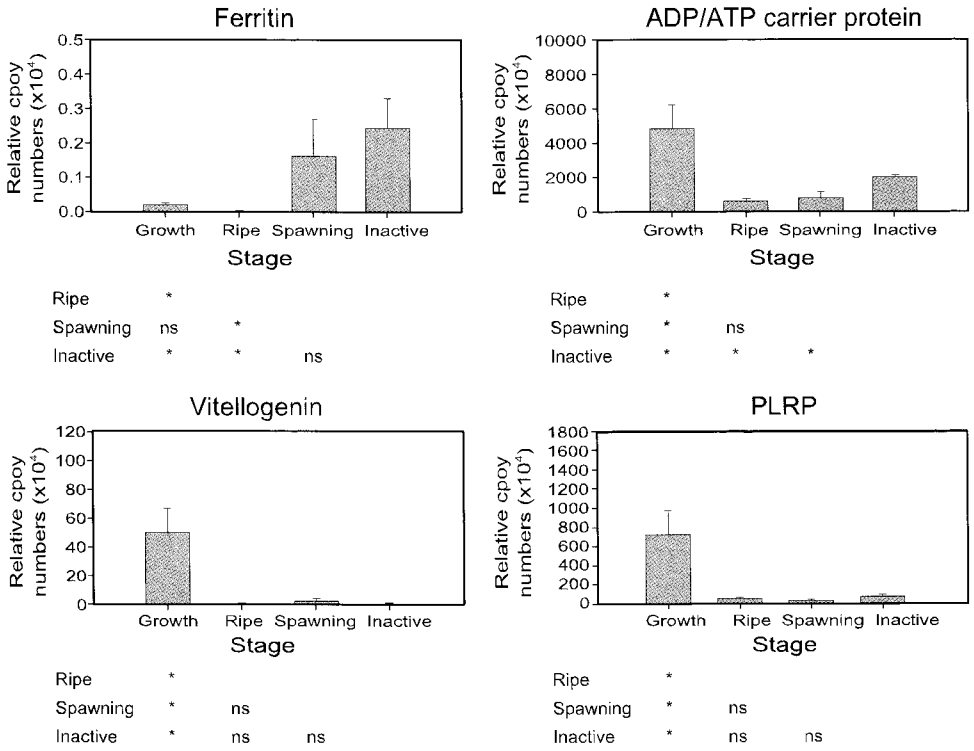

Fig. 4. Relative copy numbers of three genes during the developmental stages in the ovary of *P. yessoensis*. Copy numbers were normalized by the number of 28S rRNA. Statistical significance was shown at the bottom of the each graph. Asterisk indicates the statistical significance ($P < 0.05$) of relative copy number among different developmental stages. Values of y axis was showed as multiplying relative copy numbers by 10^4 for recognizing differences of relative copy numbers among stages easily.

Table 3. Relative copy numbers $\times(10^4)$ of three genes during reproduction cycle

	Ferritin	ADP/ATP carrier protein	Vitellogenin	PLRP
Growth Stage	0.022 (± 0.0044)	4,820.110 (± 1,368.863)	50.315 (± 16.465)	718.062 (± 248.762)
Ripe Stage	0.003 (± 0.0004)	614.338 (± 167.090)	0.226 (± 0.044)	50.381 (± 11.578)
Spawning Stage	0.162 (± 0.1098)	802.877 (± 373.688)	2.580 (± 1.895)	27.942 (± 9.474)
Inactive Stage	0.244 (± 0.0869)	1,992.589 (± 128.675)	0.277 (± 0.150)	69.489 (± 24.877)

in the other stages. In addition to those three genes, the transcript of pancreatic lipase-related protein, Py-PLRP was also checked to compare its expression level with Vg. Py-PLRP has already been identified as the ovary-specific gene in previous study (Kim et al., 2008). Interestingly, expression pattern of both Vg and Py-PLRP were synchronized suggesting both may be involved in maturating oocytes (Fig. 4). The results indicate that Vg and PLRP are good molecular marker for growth stage and play important roles in maturating oocytes.

Discussion

Three Ovary-specific genes

In present study, we used the cost-effective strategy to isolate genes involved in maturating oocytes and found three genes were transcriptionally controlled in different reproduction stages. Our result found that Vg was expressed predominantly in ovarian tissue reconfirming auto-synthetic vitellogenisis in scallop. A recently study determined total 418 sequences as EST project and seven genes were significantly expressed high in gonads of bay scallop, *Argopecten purpuratus* (Boutet et al., 2008). The result showed that two genes encoding cytidine deaminase (CDA) and vitellogenin were predominantly expressed in oocyte, whereas Testis-specific serine/threonine kinase 2 (TSSK2) and Spermatogenesis and centriole associated 1 (SPATC1) turned out to be testis-specific genes. One of transforming growth factor-β superfamily (TGF-β) member was also isolated from the gonad of oyster *Crassostrea gigas* (Fleury et al., 2008) its expression was identified both in oocyte and testis. Most recently, pancreatic lipase related protein, Py-PLRP, was also identified from *P. yessoensis* (Kim et al., 2008). Collectively, three genes appear to be ovary-specific in bivalve species until now; Vg, PLRP, and CDA. Interestingly, all those three genes are upregulated during growth stage, which suggests that they play important roles in maturating oocytes.

Vg is one of the most widely known as the precursor protein of yolk protein, which is accumulated in oocyte during maturation period in oviparous animals. Although its accumulating pattern in oocytes is similar, the sites synthesizing Vg appear to be different among phyla. Liver is the major site in all vertebrates whereas no clear conclusion has been established in invertebrate. Generally, primitive organs corresponding to the liver in vertebrates are believed to be the major site for Vg production in arthropod. Interestingly, the fat body is the only site for Vg production in insects (Sappington and Raikhel, 1998) but some decapod crustaceans may have dual production sites of Vg. For example, Vg is exclusively expressed in hepatopancreas in the suborder of Pleocyemata including Caridean species (Okuno et al., 2002; Tsutsui et al., 2004) and Astacidean species (Abdu et al., 2002), whereas both the hepatopancreas and ovaries are the sites of Vg production in the suborder of Dendrobranchiata including penaeid shrimps (Raviv et al., 2006; Tiu et al., 2006; Tsutsui et al., 2000). In contrast, autosynthetic york formation is generally accepted in bivalves. The histochemical studies found that follicle cells in the ovary appear to be the site of expression of Vg gene in bivalves including oyster and scallop (Matsumoto et al., 2003; Osada et al., 2004b). Autosynthesis of yolk protein in the ovary are also observed in some gastropods (Matsumoto et al., 2008) whereas heterosynthetic Vgs are generally accepted in most gastropods and cephalopods. Our result showing production accompanied by oocyte maturation suggests that this gene can be used as the sensitive molecular marker to estimate physiological condition. Vg production is under control of the estrogen receptor pathway in vertebrates. In fish species, Vg is synthesized in the liver in response to circulating estrogens and transported by blood stream to the ovary where it is taken up by oocytes. To be deposited as yolk protein complex, Vg is cleaved into the final egg-yolk proteins and conjugated with other lipid compounds (Wallace and Selman, 1978). Since estrogen receptor homolog was also found in bivalve, steroids may be important hormonal component in bivalve reproduction (Matsumoto et al., 2007). From those facts, Vg expression would be also one of good target genes for investigating the toxicological effect of steroid-like endocrine disrupting chemicals (EDCs) on bivalve.

PLRP was also expressed predominantly only in ovary and its expression is highest in the growth stage (Kim et al., 2008). Since its expression pattern is similar to Vg and its biological function is releasing free fatty acids from either triglycerides or phospholipids for cellular uptake (Wong and Schotz, 2002), PLRP may participate in delivery of lipids to ovarian follicles. A morphological study suggests that follicle cells encompassing a vitellogenic oocyte might transfer metabolite to the oocyte by pinocytosis in bivalves (Gaulejac et al., 1995). From those facts, PLRP is also excellent candidate for estimating physiological condition of maturating bivalve.

In addition to our study, CDA also appears to be the ovary-specific gene. CDA is structurally similar to the RNA-editing enzyme APOBEC-1, which

deaminates cytidine to uracil at specific sites of the apolipoprotein B mRNA creating a truncated protein (Wedekind et al., 2003). While the function of CDA in oocyte maturation remains uncertain, it may be involved in chromatid exchange during crossingover during meiotic cell division (Hunt and Hassold, 2002). In support of this, overexpression of mammalian CDA results in enhanced mutagenesis mimicking somatic hypermutation (SHM) in different cell types (Petersen-Mahrt et al., 2002; Yoshikawa et al., 2002). However, further study about its expression pattern in each reproduction stage and major producing tissues is necessary. Investigation of these three gonad-specific genes and associated signaling pathways will help to expand our knowledge of biochemical process in oocyte reproduction.

Roles of ubiquitously expressed genes

In addition to ovary-specific genes, we also isolated two ubiquitously expressed genes, Ft and ACC. Ft is a major iron storage protein and plays a crucial role in iron metabolism. Although iron is a constituent of metalloproteins including many enzymes and oxygen carriers, free iron, however, can be highly toxic to cells. Ft protects cells from the damaging effects of iron. Generally, Ft is involved in oxidative stress but higher level in the ovary during spawning and inactive stage may be involved in recovery from spawning as either immune response or cell cycle progression in bivalve. While echinoderm Ft acts as a acute phase protein responding to a nonlethal injury (Beck et al., 2002), Ft is also known as a gene regulator in cell cycle in other study (Le and Richardson, 2002). In fact, similar expression profiles found in testis (data not shown) suggests a common mechanism may be involved in high level of Ft expression may exist in both sex in bivalve.

ACC is the membrane protein which imports ADP and export ATP. Mitochondria are multitasking organelles which play fundamental roles including ATP synthesis, reactive oxygen species (ROS) production, and programmed cell death called Apoptosis (Ramalho-Santos et al., 2009). For successful achievement of those complicated cellular functions, the transport of various metabolites across the mitochondrial membranes is essential. Especially the exchange of ADP and ATP generates vital energy for the survival. Mitochondrial dysfunction ends up with diminished fertility, and be the cause of development retardation and arrest in human preimplantation embryos (Fissore et al., 2002; Thouas et al., 2004). Increased number of ACC mRNA transcripts may be due to the high number of mitochondrion of maturating oocyte as there is an amplification in mitochondrial number in parallel with cytoplasmatic volume increase (Jansen and de Boer, 1998). Since mitochondria of embryo are exclusively derived from the oocyte, much higher numbers of mitochondria are needed in maturating oocytes (Cummins, 2000). Further study should be carried out to understand functions in oocyte developmental process.

In addition to those two proteins, other proteins were also known to be involved in calcium signaling pathway. The most widely known genes are calcineurin A (CNA) gene and Centrin (Boutet et al., 2008; Uryu et al., 2000). CNA is catalytic subunit of serine/threoinine-specific protein phosphatase 2B, whereas CNB is the regulatory subunit (Klee et al., 1998). Higher expression of calcineurin was detected in testis than in ovary and its expression was correlated to the maturation cycle. Centrin, also known as caltractin, is a calcium-binding phosphoprotein found in the centrosome of al eukaryotic cells. It is required for duplication of centrioles and may also play a role in severing of microtubules by causing calcium-mediated contraction (Salisbury et al., 2002; Wolfrum, 1995). This suggests that centrin may be involved in chormosome structure during meiosis in reproduction cycle but its precise role and cellular functions remains to be determined.

Collectively, we isolated and characterized one gonad-specific and two ubiquitous genes, which are thought to play important roles in reproduction cycle of scallop. Further study of the genes will not only expand our knowledge of reproduction mechanism but also have many applications in aquaculture industry including increase in seed production or the year-round stable production of healthy seed.

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