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Dynamics of DNA Methylation and DNMT Expression During Gametogenesis and Early Development of Scallop *Patinopecten yessoensis*

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Abstract

DNA methylation reprograms during gametogenesis and embryo development, which is essential for germ cell specification and genomic imprinting in mammals. Corresponding process remains poorly investigated in molluscs. Here, we examined global DNA methylation level in the gonads of scallop *Patinopecten yessoensis* during gametogenesis and in embryos/larvae at different stages. DNA methylation level fluctuates during gametogenesis and early development, peaking at proliferative stage of ovary, growing stage of testis, and in blastulae. To understand the mechanisms underlying these changes, we conducted genome-wide characterization of DNMT family and investigated their expression profiles based on transcriptomes and in situ hybridization. Three genes were identified, namely PyDNMT1, PyDNMT2, and PyDNMT3. Expression of *PyDnmt3* agrees with DNA methylation level during oogenesis and early development, suggesting PyDNMT3 may participate in de novo DNA methylation that occurs mainly at proliferative stage of ovary and testis, and in blastulae and gastrulae. *PyDnmt1* expression is positively correlated with DNA methylation level during spermatogenesis, and is higher at maturation stage of ovary and in 2–8 cell embryos than other stages, implying possible involvement of PyDNMT1 in DNA methylation maintenance during meiosis and embryonic development. This study will facilitate better understanding of the developmental epigenetic reprogramming in bivalve molluscs.

Keywords DNA methylation · DNMT · Gametogenesis · Early development · Scallop

Yangping Li and Lingling Zhang contributed equally to this work.

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Introduction

DNA methylation is one of the most important epigenetic modification that widely exists in eukaryotes. It is involved in the regulation of gene expression in various biological processes. One of the most interesting phenomenon is that DNA methylation pattern undergoes profound change in gametogenesis and embryogenesis in mammals. Specifically, two cycles of DNA methylation reprogramming have been characterized (Kafri et al. 1992; Santos et al. 2002; Wolf 2007). During germ cell development, DNA methylation pattern in PGC would be erased, and different methylation patterns were written in sperm and oocyte (Seisenberger et al. 2012). This process has been proved to be necessary for X chromosome inactivation and genome imprinting (Adrian 2002; Allegrucci et al. 2005). Epigenetic modifications established during gametogenesis not only regulate gene expression in gametes, but also have influences in the zygote, embryo, and postnatal life (Stewart et al. 2016). The second phase of methylation

reprogramming occurs between fertilization and formation of the blastocyst. After fertilization, the paternal genomic DNA is actively demethylated before replication starts, and the maternal genomic DNA is passively demethylated during every cell cleavage between the 2-cell and morula stage. Methylation levels in the murine preimplantation embryo are typically at their lowest by the morula stage, and as the blastocyst is formed, they are restored after implantation (Dean and Ferguson 2001; Santos et al. 2002; Stefanie et al. 2013). Failure in this process has been demonstrated to be fatal in mice (Howell et al. 2001; Surani 2001).

DNA methylation is established through the activities of specific enzymes, DNA methyltransferases (DNMTs). In vertebrates, five DNMT genes (DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L) have been identified, of which four have been demonstrated to play vital roles in the epigenetic reprogramming during gametogenesis and early embryo development in mammals. Specifically, DNMT3A and DNMT3B are essential for de novo methylation and maintenance of DNA methylation pattern (Chen et al. 2003; Okano et al. 1999). DNMT3L could cooperate with DNMT3A and DNMT3B to establish maternal imprint (Kenichiro et al. 2002). DNMT1 plays an important role in maintenance of parental methylation imprints in early embryogenesis (Branco et al. 2008; Ewa et al. 2009).

Molluscs represent the second largest group of animals behind arthropods. Bivalvia is a class of marine and freshwater molluscs having shells composed of two valves. It includes oysters, scallops, clams, mussels, and cockles, of which many are important aquaculture animals. Due to their economic importance, previous studies in bivalves generally focused on the genes or transcriptomes related to growth (Feng et al. 2014; Hu et al. 2010), reproduction (Li et al. 2018; Li et al. 2016), and immunity (Fu et al. 2013; Li et al. 2015). In recent years, whole-genome sequencing of several bivalves such as Pacific oyster *Crassostrea gigas*, Yesso scallop *Patinopecten yessoensis*, and Zhikong scallop *Chlamys farreri* have been accomplished (Li et al. 2017; Wang et al. 2017; Zhang et al. 2012), which provides valuable resources for gene expression regulation studies. Recent advances in DNA methylation show that bivalves have some interesting DNA methylation patterns different from mammals and insects. For example, bivalves possess moderate DNA methylation level (Sun et al. 2014; Wang et al. 2014), and the methylation is enriched in intragenic region and is positively correlated with gene expression (Gavery and Roberts 2013; Gavery and Roberts 2014; Olson and Roberts 2014). Recent studies have revealed that DNA methylation is crucial for the early development of Pacific oyster (Riviere et al. 2013; Riviere et al. 2017). But till now, there still lacks a thorough investigation on the dynamics of DNA methylation during gametogenesis.

Yesso scallop *P. yessoensis* is a dioecious bivalve mollusk that distributes along the far eastern Asian coast. It reaches sexual maturation at 1 year old, and adult scallop goes through a gametogenic cycle each year. The gametogenic cycle can be divided into four major stages, including resting, proliferative, growing, and maturation stage (Yu et al. 2017). In this study, we measured the dynamics of global DNA methylation level during gametogenesis and early development of Yesso scallop, and investigated the relationship between DNA methylation level and expression patterns of DNMT genes, which is valuable for a better understanding of the epigenetic reprogramming in bivalve molluscs.

Materials and Methods

Sample Collection

To collect gonads at different developmental stages, 2-year-old Yesso scallops were obtained every month for a year from Dalian Zhangzidao Fishery Group Corporation (Liaoning Province, China). The gonads were dissected and the majority of them were frozen in liquid nitrogen for DNA and RNA extraction. The remaining part was fixed in 4% paraformaldehyde for 12–24 h and stored in 70% ethanol for paraffin sectioning.

To obtain embryonic and larval materials, artificial fertilization and larval culture were performed according to the procedure described in Wang and Wang (2008). Briefly, to induce spawning, sexually mature scallops were exposed to the air in darkness for 1 h, and then thermal stimulated by raising the seawater temperature from 9 to 12 °C. After fertilization, the embryos and larvae were reared at 13–15 °C. Embryos (2–8 cells, blastulae and gastrulae) and larvae (trochophore larvae, D-stage larvae, early-, middle-, late-pediveliger larva, and juvenile) were sampled according to the time provided by Wang et al. (2017). All experimental protocols concerning animals were conducted in accordance with the regulations of the Ethical Committee of Experimental Animal Care, Ocean University of China.

Histological Analysis

To determine the sex and stage of the gonads, histological analysis was conducted prior to DNA methylation and gene expression analysis. Before sectioning, samples were dehydrated in an ascending series of ethanol solutions, cleared in xylene and imbedded in paraffin. Each gonad sample was sectioned at 7 μm thickness. Serial sections were tiled on glass slides, deparaffinized in xylene, rehydrated in descending series of ethanol solutions and finally observed under a Nikon's Eclipse E600 research microscope. The gonads are classified into four stages, namely resting, proliferative, growing, and maturation stage according to the features described by Yu et al. (2017).

DNA Methylation Quantification

Genomic DNA was extracted from frozen tissue using a standard phenol-trichloromethane method. Then, approximately 100 ng of genomic DNA was submitted to 5-methylcytosine (5-mC) fluorimetric ELISA using the Methylflash Methylated DNA Quantification Kit (Epigentek, New Jersey, USA) according to the manufacturer's instructions. The absolute amounts of 5-mC in the samples were resolved versus a 5-mC standard curve established in parallel in the same assay. Three biological replicates that were sampled from different broodstock pools were evaluated for each developmental stages of ovary and testis.

Identification and Phylogenetic Analysis of DNMT Genes

Protein sequences of different DNMTs from *Homo sapiens*, *Gallus gallus*, *Xenopus laevis*, *Danio rerio*, *Branchiostoma floridae*, *Strongylocentrotus purpuratus*, *Apis mellifera*, *Drosophila melanogaster*, and *Nematostella vectensis* were downloaded from Uniprot (https://www.uniprot.org/) or NCBI (https://www.ncbi.nlm.nih.gov/).

To identify potential DNMT genes in Yesso scallop, those protein sequences were used to search against the predicted protein sequences of scallop genome (GenBank assembly accession: GCA_002113885.2) using local BLASTP with the parameters of "−a 16 −v 10 −b 20 −e 1e-6." The DNA methylase (DM) domain of those genes were characterized using SMART (http://smart.embl-heidelberg.de/) and aligned using ClustalX with default parameters. Phylogenetic tree was constructed by PhyML using appropriate model and parameters selected by Protest (−m LG + I + G + F −v 0.015 −a 1.431). The DNMT gene of *Schizosaccharomyces pombe* was chosen as outgroup to root the tree. The accession numbers used in the phylogenetic analysis are HsDNMT1(P26358), HsDNMT2(O14717), HsDNMT3A(Q9Y6K1), HsDNMT3B(Q9UBC3); GgDNMT1(Q92072), GgDNMT2(Q4W5Z2), GgDNMT3A(Q4W5Z4), GgDNMT3B(A0A1D5PJJ1); XlDNMT1(P79922); DrDNMT1(F1QT07), DrDNMT2(Q588C1), DrDNMT3AA(F1R1K5), DrDNMT3AB(B8JLQ7), DrDNMT3BA(B3DJN5), DrDNMT3BB1(Q588C6), DrDNMT3BB2(Q588C7); BfDNMT1(EEN54323.1); SpDNMT1(XP_011667182.1), SpDNMT3(XP_787412.3); PyDNMT1(XP_021355294.1), PyDNMT2(XP_021368212.1), PyDNMT3(MH828713); AmDNMT1(A0A088A7M4), AmDNMT2(A0A088AR60), AmDNMT3(D7RIF7); DmDNMT2(Q9VKB3); NvDNMT1(XP_001626663.1), NvDNMT3(EDO34131.1); and ScDNMT(NP_595687.1).

DNMT Gene Expression Analysis

Expression of DNMT genes during gametogenesis and early development was quantified using the transcripts per million (TPM) value from RNA-Seq data (Wang et al. 2017; Li 2018). Briefly, the high-quality reads were mapped to the *P. yessoensis* genome with STAR (Dobin et al. 2013). Raw counts for each gene were obtained by HTseq (Simon et al. 2015), and TPM were calculated using a homemade Perl script that can be found at https://github.com/YangpingLi/Transcriptome.

In Situ Hybridization

To prevent cross-detection of other DNMT genes, cDNA fragments avoiding conserved domains shown in Fig 2 were amplified with specific primers (Table 1) containing sense Sp6 promoter sequence (5′-ATTTAGGTGACACTATAG-3′) and anti-sense T7 promoter sequence (5′-TAATACGACTCACTATAGGG-3′). Purified PCR products were used as templates for in vitro transcription. Along with DIG RNA Labeling Mix (Roche, Mannheim, Germany), Sp6 and T7 RNA polymerase (Thermo, Waltham, MA, United States) were used for generating digoxigenin-labeled sense and anti-sense probes, respectively. Sections of the gonadal tissues were serially rehydrated in PBST (phosphate-buffered saline plus 0.1% Tween-20) and digested with 2 μg/ml proteinase K at 37 °C for 15 min. After pre-hybridization at 60 °C for 4 h, hybridization was performed with 1 μg/ml denatured RNA probe in hybridization buffer (50% formamide, 5 × SSC, 100 μg/ml yeast tRNA, 1.5% blocking reagent, 0.1% Tween-20) at 60 °C for 16 h. Then, the probes were washed away, and antibody incubation was performed in a fresh solution of anti-digoxigenin-AP Fab fragments (Roche, Mannheim, Germany) coupled with blocking buffer (diluted 1:2000) at 4 °C for 16 h. After extensive washing with maleic acid buffer (0.1 M maleic acid, 0.15 M NaCl, 0.1% Tween-20, pH = 7.5), sections were incubated with nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) substrate solution and counterstained with 1% neutral red solution.

Statistical Analysis

The differences among developmental stages in the gonads were detected by one-way ANOVA followed by Duncan's post hoc pairwise comparisons. To compare the differences between sexes, independent t test was performed. Differences were considered significant if p value < 0.05. Pearson's correlation coefficient was calculated to explore the relationship between *PyDnmts* expression and DNA methylation level, and significant correlation was defined when p value < 0.05.

Table 1 Sequences of primers used for *in situ* hybridization

Gene	Primer sequence (5'-3')
PyDnmt1	F: ACGACTTGAAGGAGGACATA
	R: GTTGGTGGTTCCGTTAGTTA
	F-Sp6: ATTTAGGTGACACTATAGACGACTTGAAGGAGGACATA
	R-T7: TAATACGACTCACTATAGGGGTTGGTGGTTCCGTTAGTTA
PyDnmt3	F: CTGTGTAAGGATTGCAAGGA
	R: CTGCCAATCTTCATGAGGAT
	F-Sp6: ATTTAGGTGACACTATAGCTGTGTAAGGATTGCAAGGA
	R-T7: TAATACGACTCACTATAGGGCTGCCAATCTTCATGAGGAT

Results

DNA Methylation Level During Gametogenesis and Early Development

Global DNA methylation level was first examined for gonads at four developmental stages (Fig 1a). According to the results, DNA methylation level in the ovary and testis is not constant during gametogenesis. It is lowest at the resting stage in both ovary ($1.05\% \pm 0.39\%$) and testis ($1.39\% \pm 0.49\%$) and reaches the peak at the proliferative stage of ovary ($2.42\% \pm 0.33\%$) while at the growing stage of testis (3.91%

$\pm 0.31\%$). Global DNA methylation level is relatively higher in the testis than ovary at all four stages, with significant differences at the growing and maturation stages ($P < 0.05$).

Further investigation on the DNA methylation level in scallop embryos and larvae (Fig 1b) showed that DNA methylation is low at 2–8 cell stage (0.71%), then reaches the highest level (6.16%) at blastula stage, and decreases to 2.50% at gastrula stage. Afterwards, the DNA methylation level is kept at relatively constant levels, ranging from 0.92 to 3.04%.

Identification and Phylogenetic Analysis of DNMT Genes in Yesso Scallop

To unravel the molecular mechanism underlying the dynamics of DNA methylation during gametogenesis and early development, we first identified DNMT genes by searching against the recently released Yesso scallop genome (Wang et al. 2017). Three genes were found, with the protein length ranging from 526 to 1586 aa, and the predicted molecular weight ranging from 58.54 to 179.19 KD. All three genes have multiple introns (7–35) in their genomic DNA, of which some are as long as 5.92 kb, resulting in long genes with the length varying from 12.14 to 46.62 kb. Detailed sequence information is shown in Table 2, and the cDNA sequences of the PyDNMT genes can be found in the GenBank database under accession numbers XP_021355294.1, XP_021368212.1, and MH828713.

Subsequently, conserved protein domains were identified for the three DNMTs (Fig. 2). All three proteins have a C-5 cytosine-specific DNA methylase domain in their C-terminal regions. One protein has an extra PWWP domain in its N-terminal region, and another contains four different domains (DMAP1-binding domain, cytosine-specific DNA methyltransferase replication foci domain, CXXC zinc finger domain, and BAH domain) locating upstream of the DM domain.

Alignment of the DM domains (Fig. S1) revealed that the three scallop DNMTs (PyDNMTs) share high sequence similarities with DNMT1 (72.69~83.26%), DNMT2 (40.72~52.95%), and DNMT3 (46.4~51.79%) from other
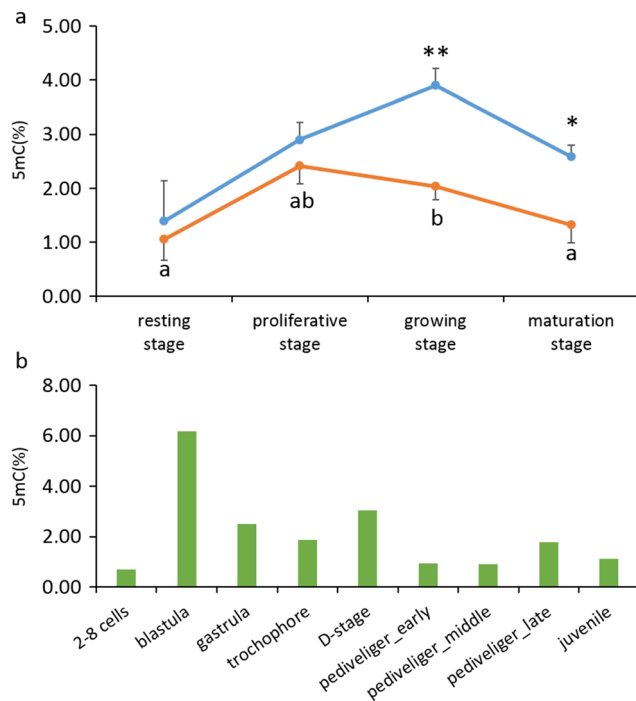

Fig. 1 DNA methylation levels during gametogenesis (**a**) and early development (**b**) in Yesso scallop. The orange and blue lines represent oogenesis and spermatogenesis, respectively. The vertical bars represent the mean ± SEM. Different letters indicate significant differences in the ovary ($P < 0.05$). The stars indicate statistically significant difference between ovary and testis (*$P < 0.05$; **$P < 0.01$)

Table 2 Basic information of the scallop DNMT gene family

Gene	gDNA length (bp)	Intron number	Protein length (aa)	DNA methylase domain position (aa)	Molecular weight (KDa)	pI
PyDnmt1	46620	35	1586	1087–1519	179.19	5.97
PyDnmt2	12793	7	526	10–507	58.54	6.74
PyDnmt3	12140	18	614	318–596	69.79	5.86

organisms, respectively. All three PyDNMTs have six highly conserved motifs in their DM domains that have been previously reported in other organisms (Kumar et al. 1994; Malone et al. 1995), suggesting scallop DNMTs may have similar functions with corresponding vertebrate DNMTs. In comparison with PyDNMT1 and PyDNMT3, PyDNMT2 seems to be more divergent from its homologs, mainly due to a 43 aa insertion at position 223 to 265. Sequence analysis showed that the insertion results from a minisatellite DNA with unit length of 48 bp repeating about 2.7 times (Fig. S2).

To confirm the identity of the three PyDNMTs, a phylogenetic tree was constructed using the DM domains of DNMT proteins from diverse organisms. As shown in Fig. 3, the selected DNMT genes were clustered into three groups, and the three PyDNMTs are classified into each of them, confirming that they are PyDNMT1, PyDNMT2, and PyDNMT3.

Expression of *PyDnmts* During Gametogenesis and Early Development

Expression of the three *PyDnmts* was investigated for the ovaries and testes during gametogenesis. As shown in Fig. 4, expression patterns of *PyDnmt1* are different between ovary and testis. In the ovary, *PyDnmt1* is low at

resting, proliferative, and growing stages, followed by a significant upregulation at the maturation stage. Expression of *PyDnmt1* is higher in the testis than ovary except for the maturation stage, and the highest level is observed at the growing stage. Relatively stable expression of *PyDnmt2* is found in the ovary throughout the gametogenic cycle, but in the testis, *PyDnmt2* seems to be higher at the resting and proliferative stages than the other two stages. Significant difference in *PyDnmt2* expression was found between ovary and testis in three stages other than the proliferative stage. *PyDnmt3* shows a similar expression pattern with *PyDnmt2* in the testis, and in the ovary it is slightly higher at the proliferative stage than the other stages. Correlation analysis showed that expression of *PyDnmt1* and *PyDnmt3* is positively correlated with DNA methylation level in the testis ($r = 0.65$, $P < 0.05$) and ovary ($r = 0.65$, $P < 0.05$), respectively.

Based on the gene expression data above, localization of *PyDnmt1* and *PyDnmt3* was conducted in the gonads at maturation and proliferative stage, respectively. Results showed that *PyDnmt1* transcripts were located in mature oocytes (Fig. 5a), spermatogonia, and spermatocytes (Fig. 5b). For *PyDnmt3*, the anti-sense probe was detected in the oogonia and oocytes of ovary (Fig.
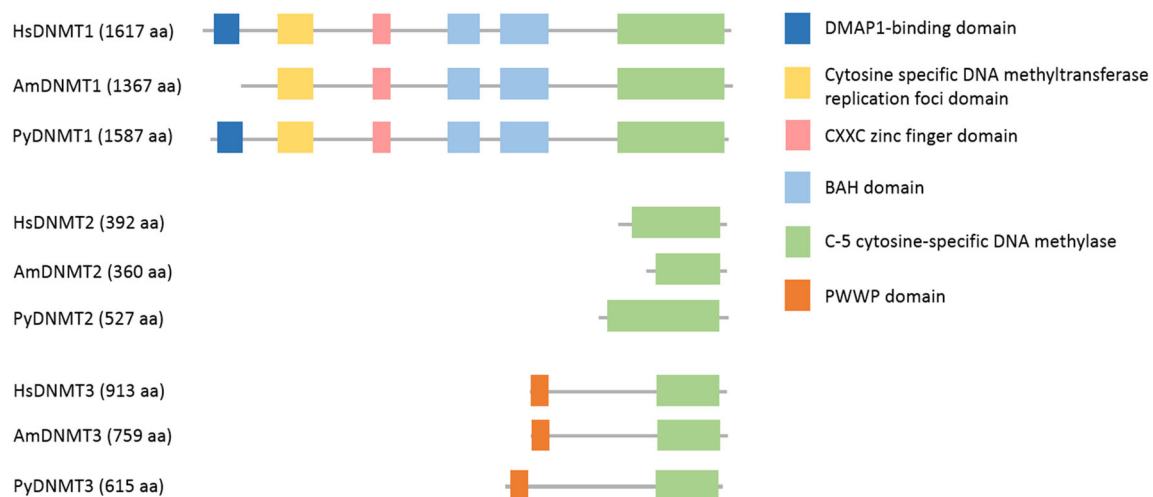

Fig. 2 Conserved domain organization of DNMT1 (**a**), DNMT2 (**b**), and DNMT3 (**c**) from *Homo sapiens* (Hs), *Apis mellifera* (Am), and *Patinopecten yessoensis* (Py). For each protein, the N-terminus is on the left and the C-terminus is on the right

Fig. 3 Phylogenetic tree of
DNMT proteins based on the
DNA methylase (DM) domains.
The maximum likelihood tree was
constructed by PhyML. Numbers
above the branches indicate boot-
strap values (200 replications).
Different branch colors denote
different DNMT groups: DNMT1
(red), DNMT2 (green), and
DNMT3 (blue). The organism
names are abbreviated as follows:
Homo sapiens (Hs), *Gallus* (Gg),
Xenopus laevis (Xl), *Danio rerio*
(Dr), *Branchiostoma floridae*
(Bf), *Strongylocentrotus
purpuratus* (Sp), *Patinopecten
yessoensis* (Py), *Apis mellifera*
(Am), *Drosophila melanogaster*
(Dm), *Nematostella vectensis*
(Nv), *Schizosaccharomyces
pombe* (Sc)
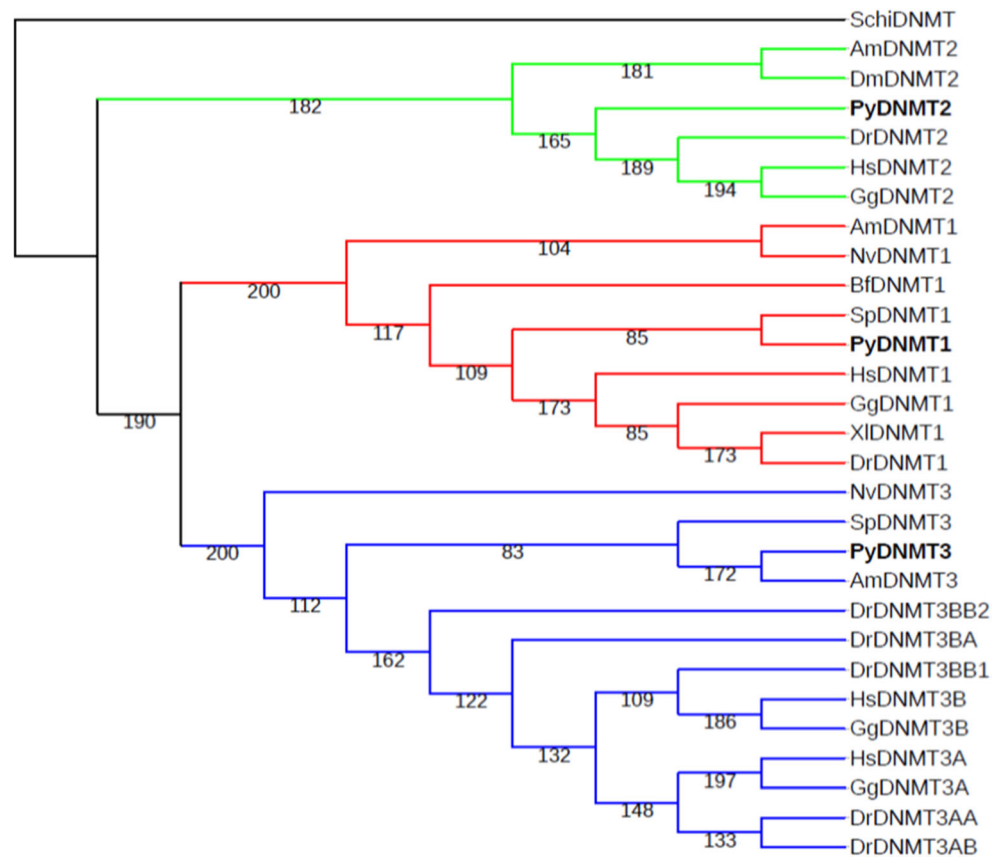
5c) and spermatogonia and spermatocytes of testis (Fig. 5d). No signal was detected in the ovary or testis with the sense probes (data not shown).

During early development, a similar expression pattern was found between *PyDnmt1* and *PyDnmt2* (Fig. 6), with the highest expression at 2–8 cell stage followed by an abrupt decrease in blastula and relatively constant levels afterwards. In contrast to *PyDnmt1* and *PyDnmt2*, *PyDnmt3* is much lower expressed and the pattern is obviously different during embryonic development. It can only be detected in blastula and gastrula stage, and there is no expression at 2–8 cell stage and during larval development. This pattern is positively correlated with global DNA methylation level during early development ($r = 0.81$, $P < 0.01$).

Discussion

DNA methylation exists widely in eukaryotes, but the extent of methylation varies considerably among organisms. According to previous reports, global DNA methylation level is approximately 0.5% in fruit fly (LePage et al. 2014), while 10–20% in human and bovine (Wiley et al. 2013; Mendonça et al. 2012). In contrast, bivalves have a relatively moderate DNA methylation level, with

4–6% in gill of mussel (Chin 2018) and 0.5–5% during early development of oyster (Riviere et al. 2013). Our data in Yesso scallop support previous studies, showing that global DNA methylation level is 2.24% ± 1.04% in the gonad and 2.12% ± 1.71% during early development. Results from global DNA methylation level agree with whole-genome bisulfite sequencing data. By calculating the proportion of methylated cytosines out of total genomic cytosines, Wang et al. (2014) reported that the methylation level of oyster mantle is 1.96%, higher than that observed in insects (0.11% in the silkworm and honeybee, 0.15% in the ant), but lower than human (3.93%). This phenomenon could result from a "mosaic" DNA methylation pattern in bivalves with accumulation of mCGs in young repetitive elements and gene bodies (Wang et al. 2014), in comparison with the "global" methylation pattern in vertebrates and limited methylation sites in insects (Schübeler 2015; Suzuki and Bird 2008).

DNA methylation of germ cells is established during the gonadal development. Differential methylation pattern in sperms and oocytes has been widely reported in vertebrates (Hisato et al. 2012; Lan et al. 2013; Smallwood et al. 2011) and insects (Remnant et al. 2016; Sylvain et al. 2012), which has been demonstrated to be essential for genomic imprinting and early
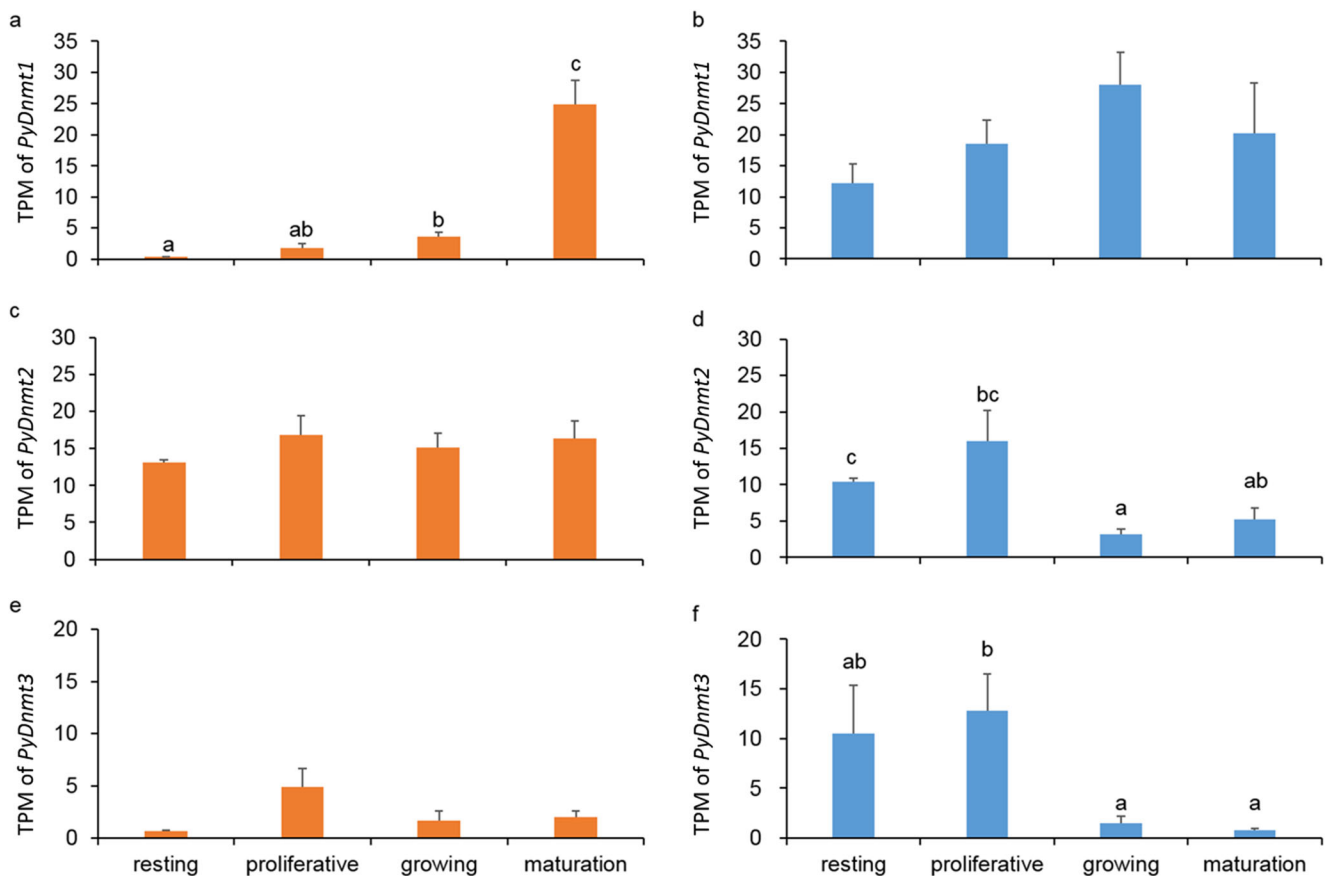
Fig. 4 Expression profiles of the *PyDnmt* genes in scallop ovaries (orange) and testes (blue) during the gametogenic cycle. **a**, **b** *PyDnmt1*. **c**, **d** *PyDnmt2*. **e**, **f** *PyDnmt3*. The vertical bars represent the mean ± SEM. Different letters indicate significant differences ($P < 0.05$)

development in vertebrates (Kenichiro et al. 2002; Reik and Walter 2001). Although DNA methylation has been characterized in oocytes and male gametes in the Pacific oyster (Olson and Roberts 2014; Riviere et al. 2013), dynamics of DNA methylation throughout gametogenic cycle has never been reported in bivalves. According to our study, different methylation levels were observed during ovary and testis development, indicating female and male gametes undergo distinct methylation modifications. Global DNA methylation level is significantly higher in the testis than ovary at the growing and maturation stages, suggesting male germ cells may be more heavily methylated than female germ cells.

DNA methylation is a critical epigenetic regulator of development not only in mammals and insects but also in some molluscs (e.g., Pacific oyster) (Riviere et al. 2013; Riviere et al. 2017). In Yesso scallop, DNA methylation levels change during early development, with a single peak at the blastula stage. This pattern is similar with that of Pacific oyster (Riviere et al. 2013), suggesting de novo methylation may occur at this particular stage. It is well known that in mammals, there is a global demethylation procedure that occurs after fertilization.

This reprogramming involves the replacement of gamete-specific methylation marks by the embryonic ones that are essential for embryonic development and the establishment of pluripotency (Hales et al. 2011; Okano et al. 1999). However, according to our results and a previous study in Pacific oyster (Riviere et al. 2013), no global demethylation process was observed. This deviation could be due to the sparse sampling from zygote to blastula. But we cannot rule out the possibility that it might be due to differences in developmental mechanisms across evolution, which would be an interesting phenomenon that deserves further research.

DNA methylation is catalyzed by DNMTs that classified into three groups (DNMT1, 2, and 3). In our study, all three genes have been identified, consistent with previous studies in Pacific oyster (Riviere et al. 2013; Wang et al. 2014). The three genes possess conserved functional domains and motifs with DNMTs from other organisms including mammals, suggesting PyDNMTs may exert similar functions to vertebrate DNMTs in epigenetic regulation. According to our results, expression profiles of *PyDnmts* are similar with *Dnmts* of Pacific oyster during early development (Riviere et al. 2013) except

Fig. 5 Localization of *PyDnmt1* and *PyDnmt3* in gonads by in situ hybridization. **a** Localization of *PyDnmt1* in the ovary at maturation stage. **b** Localization of *PyDnmt1* in the testis at maturation stage. **c** Localization of *PyDnmt3* in the ovary at proliferative stage. **d** Localization of *PyDnmt3* in the testis at proliferative stage. Og, oogonium; Oc, oocyte; MO, mature oocyte; Sg, spermatogonium; Sc, spermatocyte; St, spermatid; Sz, spermatozoon
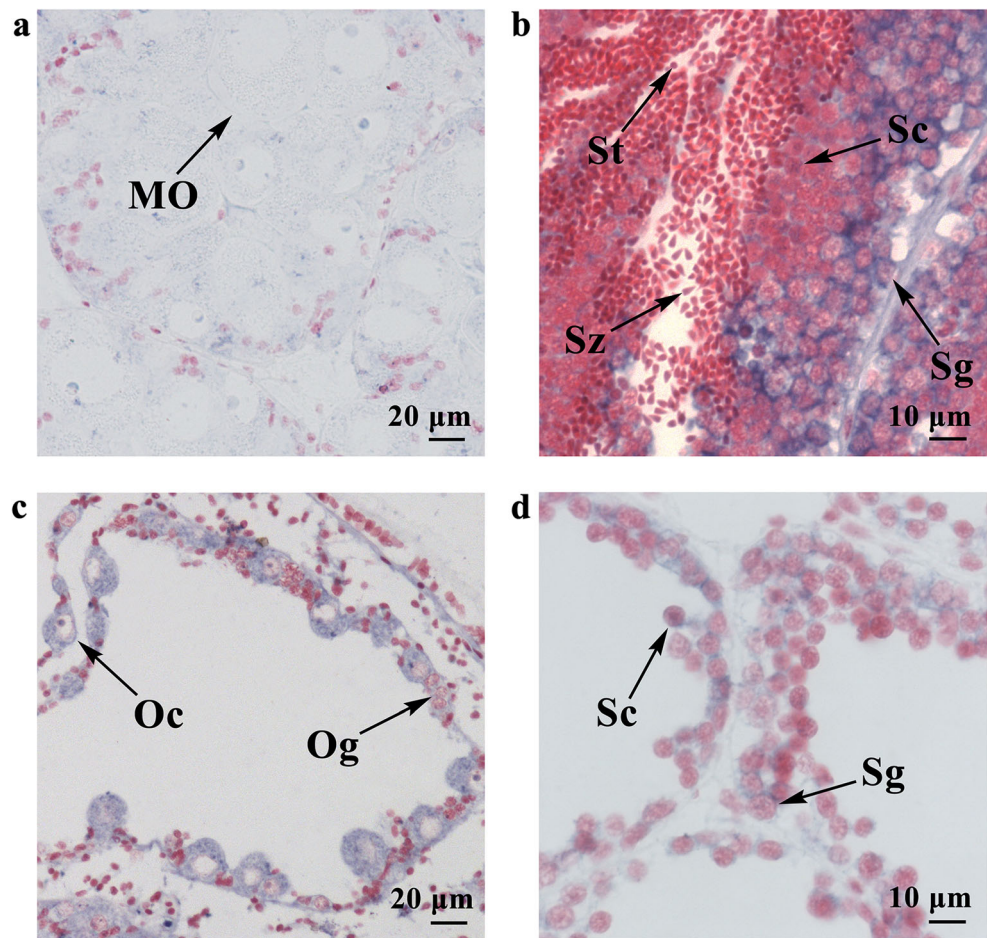
that the peak of *Dnmt3* was observed at blastula stage in scallop but at gastrula stage in oyster. Consistent with *PyDnmt3* expression, DNA methylation level peaks at blastula stage. It suggests that PyDNMT3 may be involved in de novo DNA methylation that mainly occurs at blastula and gastrula stages. *PyDnmt3* also increases at the proliferative stage of ovary and testis, and the signals located in oogonia, oocytes, spermatogonia and spermatocytes, suggesting de novo methylation may occur in these cells. Interestingly in mice, de novo methylation occurs during spermatogenesis, mainly in spermatogonia and spermatocytes in early prophase I (Kumar et al. 1994; Oakes et al. 2007), indicating this process may be conserved between vertebrates and invertebrates.

PyDnmt1 is high throughout spermatogenesis but only in mature ovary. Consistent with RNA-Seq data, the signals of *PyDnmt1* are present in mature oocytes and various germ cells in the testis. Considering that meiosis occurs during spermatogenesis and mature scallop oocytes are arrested at the first metaphase of meiosis, we assume that PyDNMT1 may be involved in DNA methylation maintenance during meiosis. Similarly in human, DNMT1 protein is present in all

spermatogenic cell stages, which was proposed to be involved in re-methylation events that might occur during meiosis and prevent methylation errors to be transmitted by the male gamete (Marques et al. 2011; Okano et al. 1998). *PyDnmt1* is of maternal source and its expression reduces as embryos develop. A similar expression pattern has been reported in the Pacific oyster (Riviere et al. 2013), implying possible involvement of DNMT1 in bivalve embryonic development. Considering that expression of *PyDnmt1* is several orders higher than *PyDnmt3*, we assume that DNA methylation maintenance could be more active than de novo methylation during early development.

PyDnmt2 exhibits a similar expression profile with oyster *Dnmt2* during early development (Riviere et al. 2013), indicating this pattern may commonly exists in marine bivalves. However, no significant relationship was found between *PyDnmt2* and DNA methylation level during gametogenesis or early development, which suggests that PyDNMT2 may not be involved in DNA methylation. This is consistent with our knowledge in other organisms that DNMT2 functions as a tRNA methyltransferase (Goll et al. 2006).
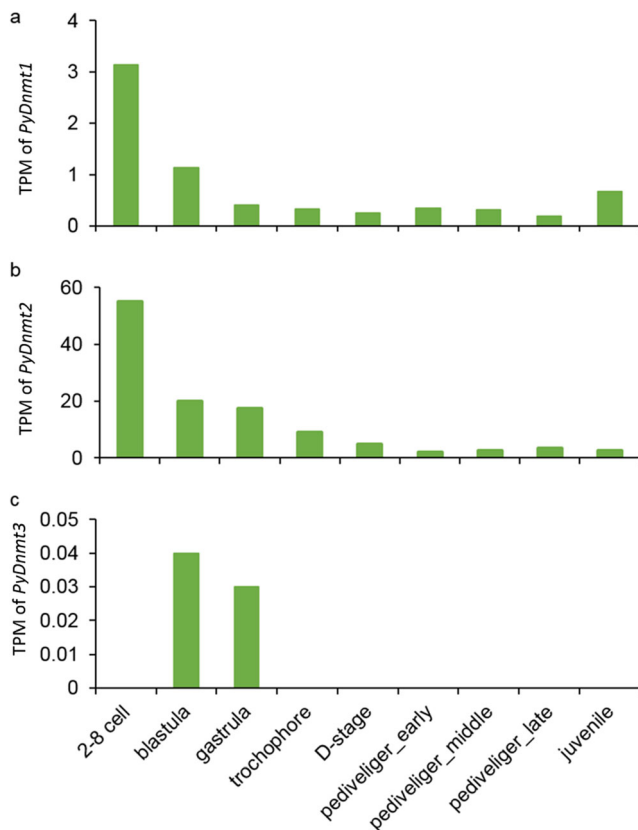
Fig. 6 Expression patterns of *PyDnmt1* (**a**), *PyDnmt2* (**b**), and *PyDnmt3* (**c**) during the early developmental stages

In summary, we examined global DNA methylation level and the expression of three Dnmts during gametogenesis and early development of Yesso scallop. Based on the results, DNA methylation levels change in both processes, in which PyDNMT1 and PyDNMT3 could be involved but exert different functions. To better understand the developmental epigenetic reprogramming, further research would be required such as what the differentially methylated regions are, and how they regulate gene expression.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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