Molecular cloning and expression analysis of *MAT1* gene in black tiger shrimp (*Penaeus monodon*)

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ABSTRACT. MAT1 (ménage à trois 1), an assembly factor and targeting subunit of the CDK-dependent kinase (CAK), can regulate the cell cycle, transcription, and DNA repair. This study was intended to investigate the role of MAT1 in the reproductive maturation of black tiger shrimp (*Penaeus monodon*). In this study, the *P. monodon* MAT1 (*PmMAT1*) gene was identified and characterized. The full-length cDNA of *PmMAT1* was 1490 bp in length with an open-reading frame of 993 bp corresponding to 330 amino acids. The temporal expression of *PmMAT1* in various tissues was measured by quantitative real-time PCR with the highest expression

observed in ovaries. In the ovaries, the *PmMAT1* gene was continuously but differentially expressed during the maturation stages. Comparative analyses of MAT1, CDK7, and cyclin H in the CAK complex of *P. monodon* indicated that the expression of *CDK7* and *cyclin H* coincided with that of *MAT1* during the ovary maturation stages. Serotonin (5-HT) injection promoted the expression level of *PmMAT1* in the ovaries of shrimp at 6-48 h post-injection. These results indicate that *PmMat1* plays a prominent role in the process of ovarian maturation.

Key words: Cloning; *PmMAT1*; Quantitative real-time PCR; 5-HT; Black tiger shrimp (*Penaeus monodon*)

INTRODUCTION

Cell division is a fundamental process in all unicellular and multicellular organisms (Korsisaari, 2002). Cell cycle progression is regulated by the sequential functions of cyclin-dependent kinases (CDKs). CDK activation requires phosphorylation of a key residue by the CDK-dependent kinase (CAK; Kaldis et al., 1998). The CAK consists of CDK7 (Levedakou et al., 1994; Wu et al., 1994), cyclin H (Fisher and Morgan, 1994), and the RING finger protein MAT1 (Fisher et al., 1995; Tassan et al., 1995; Yee et al., 1995). CDK7 is activated by cyclin H, whereas MAT1 modulates the substrate specificity of the complex (Yankulov and Bentley, 1997). MAT1 was originally identified as an assembly factor promoting a stable interaction between CDK7 and cyclin H, increasing the activity of CDK7-Cyclin H complex (Devault et al., 1995; Fisher et al., 1995; Tassan et al., 1995). CAK controls cell cycle progression by catalyzing T-loop phosphorylation of CDKs (Nigg, 1996; Kaldis, 1999), and regulates transcription by acting as a kinase of general transcription factor TFIIH to phosphorylate the carboxyl-terminal domain (CTD) of the largest subunit of RNA polymerase II (Hoeijmakers et al., 1996; Kaldis, 1999).

In vitro, the mammalian CAK has been shown to phosphorylate and activate CDK1, CDK2, CDK3, CDK4, and CDK6 (Kaldis, 1999). MAT1 modulates CAK substrate specificity in the regulation of G1 exit (Wu et al., 2001). Deletion of MAT1 by retrovirus-MAT1 antisense decreases CAK phosphorylation of pRb and induces G1 arrest in osteosarcoma cells (Wu et al., 2001). In the mouse heart, abrogation of MAT1 led to a decrease in CDK7 activity and to a general defect in transcriptional co-activation by PGC-1 family members (Sano et al., 2007). MAT1-deficient mice exhibit peri-implantation lethality; the homozygous mutant blastocysts fail to maintain/expand the inner cell mass in culture (Rossi et al., 2001). Postnatal deletion in the testis results in the loss of spermatagonial stem cells, whereas cardiac-specific MAT1 mutants develop heart failure secondary to mitochondrial dysfunction (Inamoto et al., 1997; Sano et al., 2007). The early embryonic lethality of MAT1-null embryos and the requirement for MAT1 in spermatagonial stem cell maintenance suggest that MAT1 modulates transcriptional programs that are essential for stem cell maintenance (Patel and Simon, 2010). Loss of MAT1 in mouse embryonic fibroblasts promotes adipogenesis by decreasing the inhibitory phosphorylation of proliferator-activated receptor γ (Helenius et al., 2009). These varying phenotypes suggest that MAT1 plays a prominent role in cell survival or transcription.

Although eyestalk ablation is practically used to induce ovarian maturation of the black tiger shrimp (*P. monodon*), such technique can also lead to an eventual loss in egg quality and death

of the spawners (Benzie, 1998). Research on the cell cycle regulation can improve the knowledge of molecular mechanisms controlling the development and maturation of ovaries/oocytes in *P. monodon* (Phinyo et al., 2014). To date, the regulation of oocyte maturation in crustaceans is poorly understood as compared to that in other oviparous animals. The present study was intended to provide theoretical support for an alternative approach to trigger the reproductive maturation of black tiger shrimp via conducting functional analysis of MAT1 (*PmMat1*). To examine the molecular mechanisms of MAT1 involvement in the ovarian (and oocyte) development of *P. monodon*, the *MAT1* gene was cloned, the expression patterns of *PmMAT1* in different tissues and ovarian mature stages were investigated, and the expression features of *CDK7* and *cyclin H* in the ovarian maturation stages were compared. In addition, the effects of 5-hydroxytryptamine (5-HT) on the expression of *PmMAT1* mRNA in the shrimp were examined.

MATERIAL AND METHODS

Experimental animals

Healthy black tiger shrimp (*P. monodon*) with wet weight 80-200 g were collected from Shenzhen Base of South China Sea Fisheries Research Institute, Guang Dong Province, China, and acclimated in the aerated seawater (salinity 30) for 3 days at 28°-30°C. Tissues including muscle, hepatopancreas, heart, gill, cranial nerve, stomach, intestines, and ovary were dissected out, and snap-frozen in RNAlater (Invitrogen, Japan) at -80°C before RNA extraction. Three shrimp in each ovarian mature stage were selected for RNA isolation from the ovary. Ovarian mature stage 1 (OV1) was considered a primordial germ cell stage, OV2 was chromatin nucleolus stage, OV3 was perinucleolus stage, OV4 was yolky stage, and OV5 was cortical rod stage (Huang et al., 2005).

For examining the effects of 5-HT on the expression of *PmMAT1* mRNA, shrimp (average body weight = 82.42 ± 4.15 g) were reared in laboratory tanks containing aerated seawater (salinity 30) and fed *ad libitum* for a week to gradually acclimatize them to the laboratory conditions. The stimulation group was performed by injecting with 5-HT (50 µg/g body weight) dissolved in 400 mM NaCl, 10 mM Tris-HCl, pH 7.5, into the first abdominal segment of each shrimp. The untreated shrimp and shrimp injected with 400 mM NaCl, 10 mM Tris-HCl, pH 7.5, were used as blank and control group, respectively. The injected shrimp were returned to seawater tanks, and three individuals from the blank, control, and stimulated group, respectively, were randomly collected at 0, 6, 12, 24, and 48 h post-injection (hpi). At each time point, the ovaries from the three individuals were collected and mixed and total RNA was extracted from them.

Total RNA isolation and first-strand cDNA synthesis

Total RNA was extracted from the examined shrimp using TRIzol (Invitrogen) reagent following the manufacturer protocol, was dissolved in DEPC-treated water and stored at -80°C. RNA concentration was determined by UV spectrophotometry. First-strand cDNA was synthesized from 2 µg total RNA by Moloney Murine Leukemia Virus reverse transcriptase (Promega, USA) at 42°C for 50 min with oligo-dT adaptor primer (5'-GGCCACGCGACTAGTAC(T)$_{16}$-3') following the manufacturer protocol. The cDNA was used as a template for polymerase chain reaction (PCR) to clone the gene.

Gene cloning and sequencing

Gene-specific primers, MAT1-F/R (5'-CGTGCCATTCCTCTTGTG-3' and 5'-GGTCTTGTT GCGTTCTCG-3'), were designed based on the expressed sequence tag to clone the partial-se-quence of MAT1 cDNA. The PCR cycling parameters were as follows: initial denaturation at 94°C for 3 min, followed by 34 cycles at 94°C for 30 s, 51°C for 30 s, and 72°C for 40 s. The last cycle was followed by 10 min extension at 72°C. The PCR product was purified by PCR purification kit (Sangon Biotech, China) and ligated to pMD18-T vector (TaKaRa Biotechnology, China). A portion of the ligation reaction product was transformed into DH5α competent cells and the positive clones were picked for sequencing (Invitrogen).

Full-length *PmMAT1* cDNA sequence was obtained by rapid amplification of cDNA ends (RACE) using BD SMART™ RACE cDNA amplification kit according to manufacturer instructions (Clontech Laboratories, USA). In 3'-RACE-PCR, PCR was performed initially with primer MSP1 (5'-CGAAACAGCAGACGAGTGGAACC-3') and UPM (0.4 µM UPX-long: 5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3' and 2 µM UPX-short: 5'-CTAATACGACTCACTATAGGGC-3'), followed by semi-nested PCR with MSP2 (5'-GAAGTGGCAGGAAGAGGAGAATG-3') and NUP (5'-AAGCAGTGGTATCAACGCAGAGT-3'). The PCR products were gel-purified, sequenced, and the resulting sequences were analyzed.

The sequence of the obtained *PmMAT1* cDNA was compared with other known sequences in the NCBI database using the BLAST program (Altschul et al., 1997). The pI value and molecular weight of the deduced MAT1 protein were examined using ProtParam (http://web.expasy.org/ protparam/). The N-glycosylation site was predicted using NetNGlyc 1.0 (http://www.cbs.dtu.dk/ services/NetNGlyc/). The Phosphorylation site was predicted using NetPhos 2.0 (http://www.cbs. dtu.dk/services/NetPhos/). Multiple-sequence alignments were performed using the CLUSTAL W program. The protein domain in the deduced MAT1 protein was predicted using SMART (http:// smart.embl-heidelberg.de/). Phylogenetic tree was constructed by the neighbor-joining method using the CLUSTAL W program by comparison with the MAT1 protein and Mega5.0 program using the UPGMA method (Kumar et al., 2001; Larkin et al., 2007). Accession numbers of MAT1 used for sequence alignment and phylogenetic analyses were as follows: *Sus scrofa* (NP_001159788.1); *Homo sapiens* (NP_002422.1); *Mus musculus* (NP_032638.2); *Picoides pubescens* (KFV61905.1); *Alligator mississippiensis* (XP_006267935.1); *Calypte anna* (XP_008499322.1); *Xenopus laevis* (NP_001080361.1); *Cynoglossus semilaevis* (XP_008305760.1); *Ictalurus punctatus* (NP_001188073.1); *Drosophila melanogaster* (NP_610605.1); *Ceratitis capitata* (XP_004537476.1); *Bactrocera dorsalis* (XP_011208383.1); *Penaeus monodon* (KP657569).

RT-PCR and tissue distribution analysis

Expression of *PmMAT1* in different tissues in the black tiger shrimp was analyzed by reverse transcriptase (RT)-PCR with primers RTMAT1-F/R (5'-CGAACAACAGAAGGAAGAAC-3' and 5'-GCAGTGAAGGTGGAAGAT-3') and EF-F/R (5'-AAGCCAGGTATGGTTGTCAACTTT-3' and 5'-CGTGGTGCATCTCCACAGACT-3'). EF-1α (GenBank accession No. KM212954.1) amplified from the cDNA template of the same individuals was included as the positive control. The thermal amplification reaction was initially carried out at 94°C for 3 min followed by a 34-cycle denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 45 s. The final extension was carried out at 72°C for 10 min. The amplification product was stained with ethidium bromide and visualized under a UV transilluminator.

Quantitative real-time PCR

Expression profiles of *PmMAT1* in different tissues and ovarian maturation stages in black tiger shrimp were analyzed by quantitative real-time (qRT)-PCR performed on an Applied Light Cycler (Roche Diagnostics, Shanghai) using SYBR green. Specific primers, QMAT1-F/R (5'-GAAGGAAGAACAGGTGAAG-3' and 5'-GTGAAGGTGGAAGATTTGG-3'), were used to amplify a PCR product of about 167 bp. EF-1α was chosen as the reference gene for internal standardization, whereas CDK7-F/R (5'-TCTTTCCTGCTGCCAGTGAT-3' and 5'-GGACAGGCTTATTGCTGAAAT-3', GenBank accession No. FE043423.1) and cyclin H-F/R (5'-CGTGAGATTGAAGGCAAGTTAGA-3' and 5'-TTCCTGCATTGGGGAAGC-3', GenBank accession No. AGP03382.1) were used for comparative analysis. A 10-μL reaction volume contained 5 μL 2X LightCycler 480 SYBR Green I Master (Roche Diagnostics), 50 ng first-strand cDNA template, and 0.2 μM each primer. The thermal profile for qRT-PCR was 95°C for 30 s followed by 40 cycles at 95°C for 5 s, 60°C for 20 s. Real-time PCR of each specimen was carried out in duplicate.

Statistical analysis

The comparative Ct method (Qiu et al., 2010) was used to analyze the expression level of the *PmMAT1* gene. The data obtained from qRT-PCR analysis were subjected to one-way analysis of variance (one-way ANOVA, SPSS 22.0). Differences were considered significant at $P < 0.05$.

RESULTS

Isolation and characterization of full-length *PmMAT1* cDNA

The full-length *PmMAT1* cDNA (GenBank accession No. KP657569) was 1490 bp in length and contained an open reading frame (ORF) of 993 bp with 5'- and 3'-UTRs of 70 and 427 bp, respectively. The isolated cDNA was predicted to encode a peptide of 330 amino acids with a calculated molecular mass of 38.9 kDa and a theoretical pI of 5.41. Bioinformatic analysis of the deduced polypeptide sequence identified several significant domains or motifs. The deduced amino acid of PmMAT1 contained a conserved RING finger domain (Cys13-Cys56), two coiled-coil regions (Glu133-Asp161, Val205-Glu238), a low complexity region (Pro266-Pro276), 4 N-glycosylation sites, 21 phosphorylation sites including 10 Ser, 1 Thr, and 10 Tyr residues (Figures 1 and 2). Signal P 4.0 analysis revealed that PmMAT1 did not contain a typical signal peptide sequence. The result of multiple-sequence alignment suggested that PmMAT1 was highly homologous with MAT1 of the different eukaryote species and shared a highly conserved RING finger domain (Figure 3). The dendrogram (Figure 4) depicted the evolutionary relationship based on the similarity of MAT1 proteins from the different species. The MAT1 of black tiger shrimp and insects were grouped in one cluster, and those of insect and vertebrate were grouped in another cluster.

Tissue expression analysis of *PmMAT1*

RT-PCR indicated that the expression levels of *PmMAT1* in different tissues of shrimp were comparable. Tissue distribution analysis further indicated that *PmMAT1* was abundantly
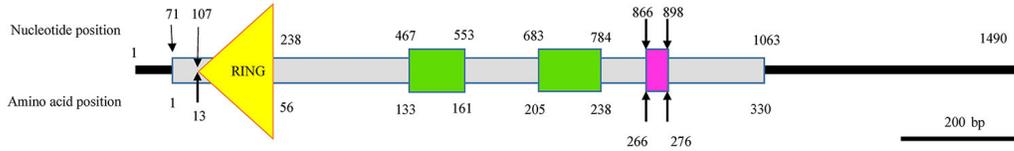
Figure 1. Schematic diagram representing the full-length cDNA of *PmMAT1*. The predicted RING domain (positions 13-56) in the deduced PmMAT1 protein is indicated by the triangle.

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1    AAACCCTTTGAGACGAAACACCAAACGTGCCATTCCTCTTGTGATATTTCTTCTTTATTA  60
61   ATATATCAGGatggggggactattacgactcctcatacgaggtccagtgccctgtgtgtaa  120
1              M  G  D  Y  Y  D  S  S  Y  E  V  Q  C  P  V  C  K   17
121  gggcacaaaatatcgtaatccacagatgaagctcatggtcaatgtctgtgggcatccaca  180
18   G  T  K  Y  R  N  P  Q  M  K  L  M  V  N  V  C  G  H  P  Q   37
181  gtgtgatgcatgtgtcagaatgaactttattaaagagtcagcacagtgttatgagtgcaa  240
38   C  D  A  C  V  R  M  N  F  I  K  E  S  A  Q  C  Y  E  C  N   57
241  cattgtacttaagcgttcaaagtttcgggtgcagatgtttgaagatccacttgtagaaaa  300
58   I  V  L  K  R  S  K  F  R  V  Q  M  F  E  D  P  L  V  E  K   77
301  ggaaatagatattagaagaaaggttatgagaattacaataaatcagaggaagatttcga  360
78   E  I  D  I  R  R  K  V  M  R  I  Y  N  K  S  E  E  D  F  E   97
361  aacagcagacgagtggaacctttatctagaagaaatagaagagatcgtctttaacatcac  420
98   T  A  D  E  W  N  L  Y  L  E  E  I  E  E  I  V  F  N  I  T   117
421  aaatgatattaaccgcttagaaatggagaagaaaattgagaactatgaacgcatgaataa  480
118  N  D  I  N  R  L  E  M  E  K  K  I  E  N  Y  E  R  M  N  K   137
481  agacgaaatcagaagaaattattccaagaagagtaaggagctggaagaaatagacaagca  540
138  D  E  I  R  R  N  Y  S  K  K  S  K  E  L  E  E  I  D  K  Q   157
541  gattgatgcagacatgatgagagaaatggaaagaaagaagtggcaggaagaggagaatga  600
158  I  D  A  D  M  M  R  E  M  E  R  K  K  W  Q  E  E  E  N  E   177
601  aagatcgaaaatatctcgagaacgcaacaagacctctcttctacaggaccttatggcatc  660
178  R  S  K  I  S  R  E  R  N  K  T  S  L  L  Q  D  L  M  A  S   197
661  agatggcgatgcgtccctcatcgtgcagtctcatgcagaacgcttgaaggtcgaacaaca  720
198  D  G  D  A  S  L  I  V  Q  S  H  A  E  R  L  K  V  E  Q  Q   217
721  gaaggaagaacaggtgaagaagaaaaagaaatattaagaagacaacaaataatggcaca  780
218  K  E  E  Q  V  K  K  E  K  E  I  L  R  R  Q  Q  I  M  A  Q   237
781  ggaattttcatctggtgtcaagattggatttgcagggattagtgctcctatcaaagttca  840
238  E  F  S  S  G  V  K  I  G  F  A  G  I  S  A  P  I  K  V  Q   257
841  gaaggaacaacagtatgagtacgagcctccaaatcttccaccttcactgccagctcccac  900
258  K  E  Q  Q  Y  E  Y  E  P  P  N  L  P  P  S  L  P  A  P  T   277
901  ctgggacgagctggaatcgggtggctaccttaaccacgtccgccgtgcttcaccttcagc  960
278  W  D  E  L  E  S  G  G  Y  L  N  H  V  R  R  A  S  P  S  A   297
961  ggtcgcaggaggatacacagaacactatgcttgtctcagggctttgcaggactttatgag  1020
298  V  A  G  G  Y  T  E  H  Y  A  C  L  R  Q  D  F  M  S   317
1021 ttctcatatgttaatacctagatctaaagaacaagaagtataaTACGATATTATGAGTAT  1080
318  S  H  M  L  I  P  R  S  K  E  Q  E  V  *               330
1081 ATAACTGGTATTTTGTATAACAATAATTTTCTGTTGGGGCTTTCAGATATTCCTCCAATT  1140
1141 TATTTAAATGATTTATGAAAGCAGTGTCTACAGAGAGGTTTAAAGTAACCACAAAACTGC  1200
1201 CAAAGAAAACACTGCGTACTTTGCGTTTCTTTTAGGTAAATTTATTTCCCGGTTAAAATA  1260
1261 TGATCCGCATGCCAGGTTTGATGTTAAGTGTACAATATACAATTACAATATACAATATAC  1320
1321 CTCAGTATCTTTCATGCAACAATGAATTTCATACAAGAATTAATATAGAGCACTTGAGAA  1380
1381 AACAAAAGGGTAAGCTTCTGTATCAGTGCTGTCCATGTTACTTTACTGGAGGCTAAAAAA  1440
1441  CAATAACAAAGTATAATTTTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA        1490
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Figure 2. Nucleotide and deduced amino acid sequence of full-length *PmMAT1* cDNA. Numbers on the left and right of each row refer to the nucleotide or amino acid position. The initiation codon (ATG) and the termination codon (TAA) are indicated in bold font. The poly A signal sequence is italicized. The RING finger domain is highlighted. Potential N-glycosylation sites are underlined.
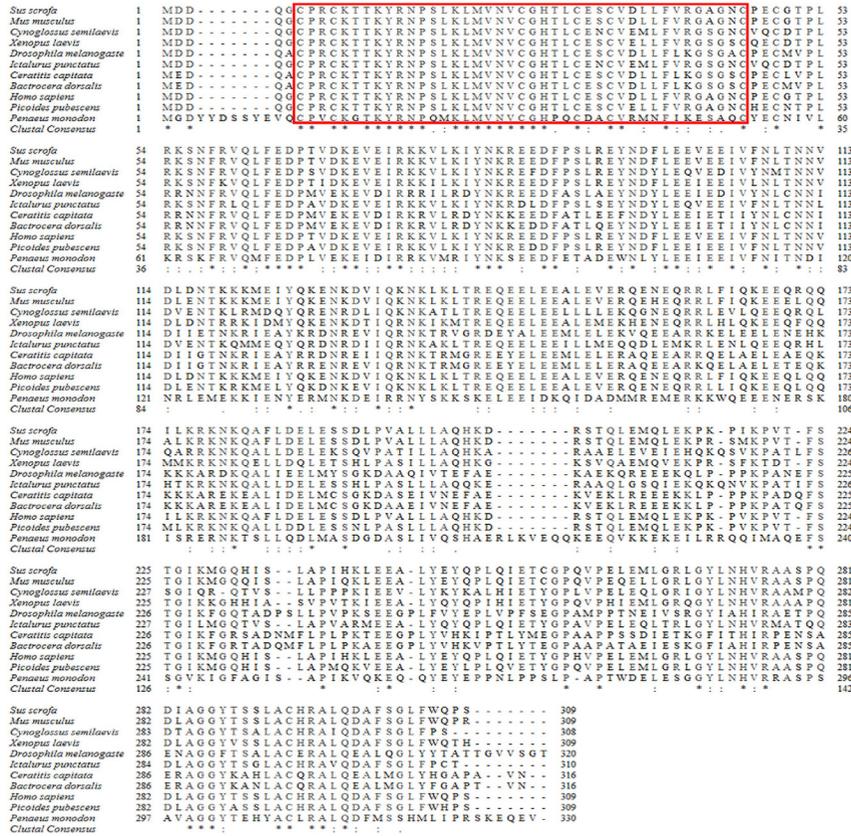
Figure 3. Multiple alignments of PmMAT1 with other known MAT1 amino acid sequences aligned using the CLUSTAL W program. Identical and similar sites are indicated with asterisks and dots or colons, respectively. Box marked in red encompasses the conserved RING finger domain in different species.
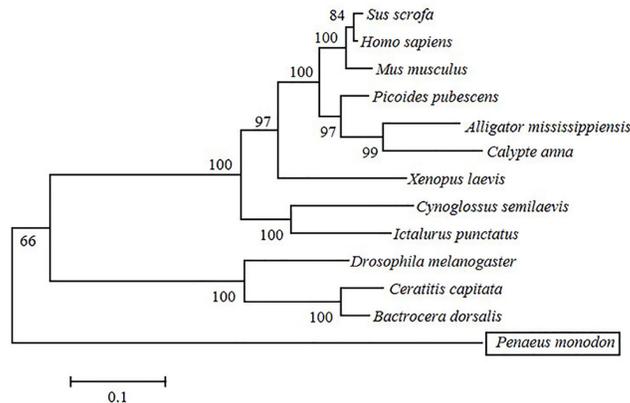
Figure 4. Bootstrapping phylogenetic tree of MAT1 proteins from different species generated by CLUSTAL W and Mega program using the UPGMA method. Values at the node represent the percentage of times the particular node occurred in 1000 trees generated by bootstrapping the original aligned sequences.

expressed in ovaries, hepatopancreas, and muscles. Low expression levels of *PmMAT1* were observed in other tissues including heart, stomach, gill, intestines, and cranial nerve of female shrimp (Figure 5).
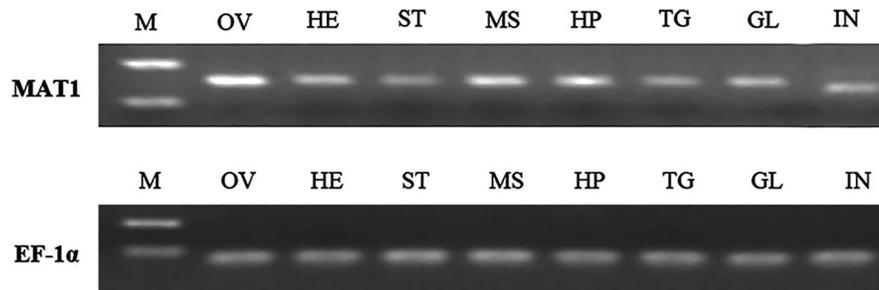
Figure 5. RT-PCR of *PmMAT1* using the first-strand cDNA from tissues of *Penaeus monodon*. Maker (M), ovaries (OV), heart (HE), stomach (ST), muscle (MS), hepatopancreas (HP), thoracic ganglion (TG), gill (GL), and intestine (IN).

Expression profiles of *PmMAT1* in *P. monodon*

Results from qRT-PCR indicated that *PmMAT1* was constitutively expressed in all the examined tissues with significant variation in the expression levels in the shrimp. The expression level of *PmMAT1* in ovary, hepatopancreas, and muscle was high whereas it was low in the heart, stomach, gill, cranial nerve, and intestines. The highest level of *PmMAT1* expression was detected in ovary, showing approximately 2-fold the expression observed in the hepatopancreas (Figure 6A). The *PmMAT1* transcripts were constitutively expressed in ovary but the expression varied during the maturation stages. The expression level of *PmMAT1* sharply increased in OV3 while it was very low in the other stages (Figure 6B).

Expression profiles of *MAT1*, *CDK7*, and *cyclin H* in maturation stages of ovaries in *P. monodon*

The expression levels of *CDK7* and *cyclin H* sharply increased in the second to the third stage and decreased in the fourth stage. Multiple comparison of the three expression characters showed that the levels of *CDK7* and *cyclin H* followed the same trend of variation as the expression features of MAT1 during the maturation stages of ovaries (Figure 6C).

Effects of 5-HT injection on *PmMAT1* transcription in ovaries of *P. monodon*

The effects of 5-HT on the expression of *PmMAT1* in ovaries of *P. monodon* were examined. The expression level of *PmMAT1* gradually increased at 6-48 hpi, and reached the highest level at 48 hpi (Figure 7).

DISCUSSION

The present study reports the cloning and characterization of a full-length *MAT1* cDNA in
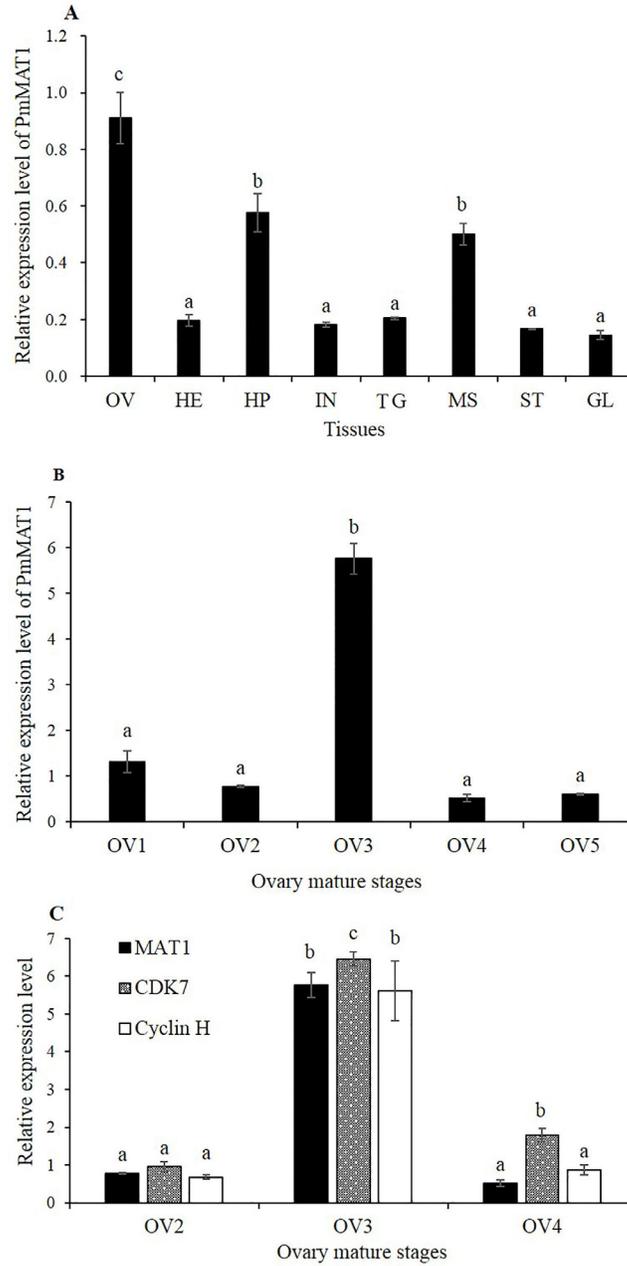
Figure 6. qRT-PCR analysis of *PmMAT1* expression levels in different tissues of *Penaeus monodon*. Relative expression levels of *MAT1* in different tissues (**A**). Relative expression levels of *MAT1* in the different maturation stages of ovary (**B**). Relative expression levels of *MAT1*, *CDK7*, and *cyclin H* in ovarian maturation stages (**C**). OV1: primordial germ cell stage; OV2: chromatin nucleolus stage; OV3: perinucleolus stage; OV4: yolky stage; OV5: cortical rod stage. Vertical bars represent means ± SE (N = 3). Bars with different letters (a, b, and c) differed significantly (P < 0.05). For tissue abbreviations, see Figure 5.
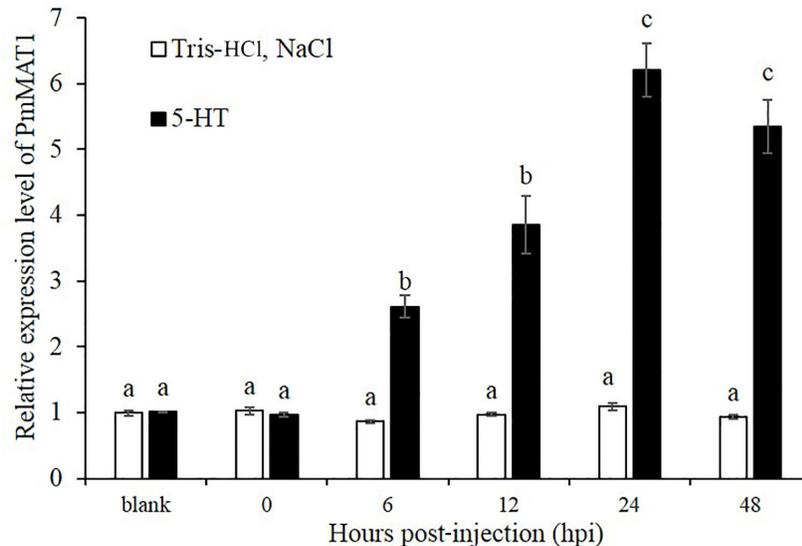
Figure 7. qRT-PCR analysis of the *PmMAT1* expression levels after 5-HT stimulation in ovary. Vertical bars represent means ± SE (N = 3). Bars with different letters (a, b, c) differed significantly (P < 0.05).

black tiger shrimp (*P. monodon*) using the RACE technique. The full length of this cDNA was 1490 bp and included an ORF of 993 nucleotides encoding a polypeptide of 330 amino acids with an estimated molecular mass of about 38.9 kDa. As shown in Figures 1 and 2, there was one RING finger domain, two coiled-coil regions, and a low-complexity region. Multiple-sequence alignment suggested that PmMAT1 was highly homologous to MAT1 of different eukaryote species and shared a highly conserved RING finger domain (Figure 3). The deduced amino acids of PmMAT1 showed moderate similarity to MAT1 from other organisms, such as *C. capitata* (positive 44%, *E*-value = 7e-74), *B. dorsalis* (positive 41%, *E*-value = 3e-73), and *P. pubescens* (positive 43%, *E*-value = 5e-82). The *E*-value were all lower than 0.005. If the *E*-value is lower than 0.005, the sequences are considered homologous (Anderson and Brass, 1998). The phylogenetic tree demonstrated that the target gene was clustered with that of arthropod (Figure 4). These conserved characteristics together proved that PmMAT1 belonged to the MAT1 family. The RING finger domain is conserved from invertebrates to vertebrates (Figure 3) and appears to be involved in transcription activation and CTD phosphorylation (Busso et al., 2000). In the MAT1-deficient trophoblast cells, MAT1 regulates the CTD phosphorylation levels *in vivo* either by affecting the activity of CDK7-Cyclin H kinase directly or by modifying the activities of the other CTD kinases (Korsisaari, 2002). The central coiled-coil motif was primarily suggested to mediate CDK7-Cyclin H-MAT1 binding with the core TFIIH through interactions with XPD and XPB (Rossignol et al., 1997; Busso et al., 2000; Sandrock and Egly, 2001). The MAT1 polypeptide in black tiger shrimp contained two coiled-coil regions while in others it contains only one. This difference in the structure implies variance in their functions. The low complexity region (LCR) may be involved in flexible binding associated with specific functions. LCR-containing proteins tend to have more binding partners across different protein-protein interaction networks than proteins that have no LCR (Coletta et al., 2010). LCRs exhibit a variety of puzzling evolutionary phenomena (DePristo et al., 2006). In vertebrate MAT1, there is no LCR, which indicates that PmMAT1 plays a novel role in the CAK complex in black tiger

shrimp. CAK regulates cell cycle progression by activating CDK complexes through phosphorylation of a critical threonine residue in their T-loop domain (Sherr, 1994; Morgan, 1995), whereas MAT1 functions in the cell cycle by stabilizing the association of CDK7 with cyclin H (Devault et al., 1995; Fisher et al., 1995; Tassan et al., 1995) and determining the substrate specificity of CDK7-cyclin H (Ko et al., 1997; Rossignol et al., 1997; Yankulov and Bentley, 1997). Therefore, our data strongly indicate that the identified MAT1 gene might be related to cell proliferation in black tiger shrimp, both mitotically and meiotically.

The development of oocytes involves a series of complex cellular events, in which different genes are expressed to ensure the proper development of oocytes and to store transcripts and proteins as maternal factors for early embryogenesis (Qiu et al., 2005; Phinyo et al., 2014). In the present study, the mRNA expression of black tiger shrimp MAT1 could be detected in all the tested tissues (Figure 5). The highest level of MAT1 mRNA expression was detected in the ovary (Figure 6A), which was consistent with the fact that there is massive cell proliferation during ovarian development and maturation. In other tissues, *PmMAT1* was abundantly expressed in hepatopancreas and muscles (Figure 6A). This could mean that PmMAT1 is related to the growth and metabolism in black tiger shrimp. Therefore, PmMAT1 may be concerned with the growth in the black tiger shrimp, and it may play a more important role in oogenesis (and ovarian development) than in other tissues of *P. monodon*. The results of genetic mutations in mouse MAT1 revealed an absolute requirement for MAT1 both in the mitotic embryonic inner cell mass and adult germ cells (Korsisaari, 2002). To further understand the possible biological function of the PmMAT1, its temporal expression pattern was quantified during the different maturation stages of ovaries by qRT-PCR. We observed that the abundance of *MAT1* mRNA increased sharply in the third stage (perinucleolus stage; Figure 6B). In the first stage, the ovary did not begin to grow; in the fifth stage, the development of the ovary began to end; and from the second to the fourth stage, the ovary was in the developmental phase. This result suggested that the cloned *PmMAT1* gene should play a critical role in development during the ovarian stages. To better understand the function of the MAT1 in the CAK complex, comparative analysis of CDK7 and cyclin H expression in the ovaries was conducted. The expression levels of *CDK7* and *cyclin H* coincided with those of *MAT1* in the maturation stages of the ovary (Figure 6C). CDK7 with its cyclin partner, cyclin H, and a third subunit MAT1 has been implicated in positive regulation of the other CDK-cyclins *in vitro* (Korsisaari, 2002). MAT1 was required to achieve the normal levels of CDK7 and cyclin H protein in cardiac muscles and to recruit the kinase complex to the core components of TFIIH (Sano et al., 2007). Deleting MAT1 in myocardium severely impaired CDK7 activity, both as a free heterodimer and as a part of TFIIH (Sano et al., 2007). Therefore, it can be concluded that the MAT1 plays a significant role in the CAK complex regulating cell cycle progression in black tiger shrimp.

The stimulation of ovarian maturation and spawning by 5-HT has been reported in *P. monodon* (Wongprasert et al., 2006). To further understand the potential function of PmMAT1 in *P. monodon*. We injected shrimp with the same amount of 5-HT and found that 5-HT enhanced the expression level of *PmMAT1* at 6-48 hpi (Figure 7). Likewise, 5-HT injection (50 mg/g body weight) resulted in the up-regulation of *PmCdc2*, which is functionally involved in meiotic maturation of oocytes, at 1 hpi (Phinyo et al., 2013) and *PmCDK7*, which is functionally important in ovarian development, fertilization, and embryo development in *P. monodon* (Phinyo et al., 2014). Results obtained in the present study confirmed the molecular effects of 5-HT on the transcription of genes functionally involved in the signal transduction and indicated that 5-HT might directly enhance the meiotic maturation of oocytes in *P. monodon* by stimulation of the MPF (a complex of Cdc2 and

cyclin B) via the activity of CAK (PmMAT1, CDK7, and cyclin H).

Loss of MAT1 is associated with the loss of TFIIH kinase activity and destabilization of CDK7 and cyclin H. The observed general transcription defect in the MAT1-deficient cells, therefore, may be partly mediated through direct decreased activation of elongation factors by CDK7 (Helenius et al., 2011). In mammalian cellular models, CAK is involved in the regulation of cell cycle G1 exit while MAT1-modulated CAK formation and CAK phosphorylation of pRb may determine the cell cycle specificity of CAK in the G1 progression (Wu et al., 2001). Manipulation of MAT1 abundance shows that MAT1 reduction mimics retinoic acid-induced hypophosphorylation of pRb/RXRα, proliferation inhibition, and neurite outgrowth, whereas MAT1 overexpression resists these retinoic acid actions, which reveal an important mechanism by which MAT1-modulated CAK activity is crucial in the switch from proliferation to differentiation in neuroblastoma cells (Zhang et al., 2004).

In conclusion, PmMAT1 might play a functional role in the development of oocytes in *P. monodon*. The basic knowledge obtained in this study will allow the characterization of the function of PmMAT1 in the control of oocyte maturation in this economically important species. However, the *MAT1* gene regulation mechanism should be explored further in a more comprehensive study.

Conflicts of interest

The authors declare no conflict of interest.

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