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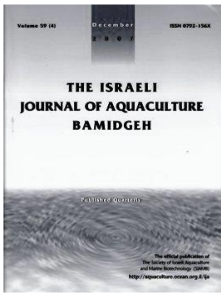

Molecular Cloning and Expression Analysis of Cyclin H from Black Tiger Shrimp (*Penaeus monodon*)

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Key words: *Penaeus monodon*, Cloning, expression, cyclin H

Abstract

Cyclin H is an important cell protein that plays a crucial role in cell division. In the present study, the cDNA of cyclin H (designated as *Pm*cyclin H) was identified from black tiger shrimp (*Penaeus monodon)* by expressed sequence tag (EST) analysis, and RACE techniques. The full length cDNA of *Pm*cyclin H is 1280bp, including a 5'-terminal un-translated region (5'UTR) of 63 bp, a 3'UTR of 218 bp with a poly (A) tail, and an open reading frame (ORF) of 999 bp encoding a polypeptide of 332 amino acids with a predicted molecular weight of 39 kDa, predicted pI of 6.39. Both Blast, and phylogenetic analysis, confirmed that *Pm*cyclin H is a new member of the shrimp cyclin H family. Using real-time PCR the mRNA expression of *Pm*cyclin H in eight tissues was examined, and mRNA transcript of Pmcyclin H was predominantly detectable in ovarian tissue, and to a lesser degree in the tissues of intestine, testis, stomach, and heart, but almost undetectable in the tissues of liver (hepar), brain, and muscle. The temporal expression of *Pm*cyclin H in different developmental stages of the ovaries was investigated by real-time PCR. During the six stages of ovarian development, one peak expression of *Pm*cyclin H was detected in stage II. All these results indicated that *Pm*cyclin H might be involved in the regulation of cell cycle and ovarian development of *P. monodon*.

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Introduction

Cyclins are the positive regulatory subunits of cyclin-dependent kinases (CDK). They play a crucial role in the coordination of the eucaryotic cell cycle by binding to the catalytic subunits of CDKs. They share distant sequence homology over a 100 amino acid region called the cyclin box, and have been classified in two phylogenetically divergent subfamilies. The first contains A, B, D, E, F, G, cyclins and the second contains C and H cyclins (Andersen et al., 1996). The activation of cycline dependent kinase (CDK) needs cyclin protein and CDK-activating kinase (CAK). CAK is important for proper replication and division of the genome. Consequently, imbalance by either overexpression, or deletion of single components is usually detrimental to the cell and the basis for malignant transformation (Krempler et al., 2005). CAK is a trimeric complex consisting of CDK7, cyclin H, and MAT1, which activates the cell-cycle-regulating CDKs through T loop phosphorylation (Krempler et al., 2005). Several control mechanisms have evolved to ensure correct activation or deactivation of CDKs. One is the activation of CDK molecules by dephosphorylation and phosphorylation of defined residues in the CDK polypeptide chain (Morgan et al., 1995). Notably, a cyclin/CDK complex itself is the core component of CAK (Fisher et al., 1994; Kaldis et al., 1999; Mäkelä et al., 1994). Cyclin H/CDK7 together with the assembly factor MAT1 activates cell-cycle-regulating CDKs by phosphorylation of a conserved threonine residue in their T loop region (Devault et al., 1995; Fisher et al., 1995; Tassan et al., 1995). Further investigations revealed that cyclin H is also a subunit of the general transcription factor TFIIH, a multi-protein complex involved in three important mechanisms: transcription, DNA repair, and cell cycle regulation, containing at least nine subunits and shown to possess several enzymatic activities including helicase, ATPase, and kinase. Besides the function of the above, cyclin H/CDK7/p36 can specifically phosphorylate distinct residues in recombinant carboxyl-terminal domain (CTD) of RNA polymerase II substrates (Feaver et al., 1994; Roy et al., 1994; Shiekhattar et al., 1995; Drapkin et al., 1996). Little is known about the regulation of the CAK complex through cyclin H especially in invertebrate animals. So the molecular cloning and characterization of the cyclin H gene is important for us to study and clarify the mechanism of cyclin H. This may assist in the clarification of the maturation mechanism of black tiger shrimp.

The black tiger shrimp (*Penaeus monodon*) is widely distributed in the Indo-West Pacific Ocean (Tassan et al., 1995; Feaver et al., 1994). In South China, black tiger shrimp is an important species for the aquaculture industry. In 2006, 79,244 metric tons of farmed *P. monodon* were produced in China (China fisheries statistics yearbook; unpublished). However, the mechanism of oocyte maturation in *P. monodon* is poorly understood. Many maturation genes such as *Pm*cyclin B, *Pm*cyclin E, PmCDK 7, etc. (Visudtiphole et al., 2009; Zhao et al., 2014; Phinyo et al., 2014) have been cloned however there have been no reports explaining the function of the cyclin H gene in development and differentiation of shrimp ovary. The purposes of the present study were: 1) to clone the full-length cDNA of cyclin H gene from black tiger shrimp *P. monodon* (*Pm*cyclin H); 2) to investigate tissue distribution of *Pm*cyclin H; 3) to check temporal expression of *Pm*cyclin H during the developmental stages of the ovaries, and 4) to promote ovarian maturation of black tiger shrimp by means of regulation of expression of *Pm*cyclin H. We hope the data presented in this study may be useful for understanding the mechanism of oocyte maturation in *P. monodon*.

Materials and Methods

Experimental animals. Healthy black tiger prawns, *P. monodon* (fresh weight 60-200g) were purchased from Guangzhou, Guangdong province, P. R. China and maintained at 25±1^{o}C in tanks for three days prior to onset of the experiment. They were used as source material for the cDNA library construction and expressed sequence tag (EST) analysis, cDNA cloning and expression analysis. For 3′ and 5′ Rapid Amplification of cDNA Ends (RACE), the RNA was isolated from the ovaries of three shrimp each weighing about 200g. For gene expression: (1) three shrimps were sacrificed prior to RNA isolation from the tissues including liver (hepar), ovary, muscle, brain stomach, heart, intestine and testis; (2) three other shrimps were sacrificed prior to RNA isolation from the ovary of six different development stages which were detected by anatomical and histological methods and classified according to the report of Huang (Huang et al., 2005). The six developmental stages of the ovaries are I: primordial germ cell stage, II: chromatin nucleolus stage, III: perinucleolus stage, IV: yolky stage, V: cortical rod stage, VI: spent stage.

cDNA library construction and EST analysis. A cDNA library was constructed from the ovary and neurosecretory organ in the eyestalk of an adult black tiger prawn, using the ZAP-cDNA synthesis kit and ZAP-Cdna GigapackⅢ Gold cloning kit (Stratagene). Random sequencing of the library using T3 primer yielded 6782 successful sequencing reactions. BLAST analysis of all the EST sequences revealed that an EST of 1084bp was homologous to the cyclin H of human.

Total RNA isolation. Total RNA was isolated from the examined tissues (weight about 50mg) of the shrimps using Trizol (Invitrogen, USA) reagent following the protocol of the manufacturer, and resuspended in DEPC-treated water and stored at -80°C.

cDNA Synthesis. cDNA was synthesized from 2μg of total RNA by Moloney Murine Leukemia Virus reverse transcriptase (M-MLV, Promega, USA) at 42°C for 50 min with oligo-dT-adaptor primer (5′ GGCCACGCGTCGACTAGTACT$_{17}$ 3′) following the protocol of the manufacturer. The cDNA was used as the template for PCR reactions in gene cloning and expression analysis.

Gene cloning and sequencing. The 3′ ends of mRNA were obtained by rapid amplification of cDNA ends (RACE) methods. In 3′ RACE-PCR, PCR reaction was performed initially with primer F1 (nucleotide position 557-577, 5′ AACCATTCCGCCCAGTAGAAG 3′) and adaptor primer (5′ GGCCACGCG TCGACTAGTAC 3′), followed by semi-nested PCR with F2 (nucleotide position 859-879, 5′ AACCTCCTGCCTCGGACACT 3′) and adaptor primer. The PCR profile was as follows: 94°C, 5 min, one cycle; 94°C, 45 s; 59.5°C, 30s; 72°C, 45s; 35 cycles; 72°C, 10 min, one cycle. The size of the fragment cloned was about 421bp. The PCR products were gel-purified, sequenced, and the resulted sequences were subjected to analysis.

Sequence analysis of Pmcyclin H. The searches for nucleotide and protein sequence similarities were conducted with BLAST algorithm at the National Center for Biotechnology Information (http://www.ncbi.nlm.gov/blast). The cyclin H amino acid sequence was predicted using DNA-Tool version 6.0 software. Analysis of the deduced amino acid sequences was conducted using the programs PSORT (Kenta Nakai, National Insitute Basic Biology), Scan Prosite (EXPASy Molecular Biology Server), and Predict Protein (EMBL-Heidelberg). The phylogenetic tree was constructed by the neighbor-joining (NJ) method using the programs of CLUSTAL X1.83 (Brady et al., 2012) and MEGA3.1 (Treerattrakoo et al., 2011).

Quantitative Real time PCR (qRT-PCR) analysis of Pmcyclin H gene expression. Real-time quantitive PCR was performed with the SYBR Green 2×Supermix (Applied Biosystems, USA) on an ABI 7300 Real-Time Detection System (Applied Biosystems, USA) to investigate the expression of *Pm*cyclin H. Two specific primers, rF (5′CGTGAGATTGAAGGCAAGTTAGA3′) and rR (5′GCTTCCCCAATGCAGGAA3′) were used to amplify a PCR product of 110 bp. β-actin (GenBank accession No. EF087977) was chosen as reference gene for internal standardization (Liu et al., 2007). Two β-actin primers ractinF (5′ATGGTTGT CAACTTTGCCCC3′) and ractinR (5′TTGACCTCCTTGATCACACC3′) were used to amplify a β-actin gene fragment of 110 bp as the internal control for qRT-PCR. The qRT-PCR amplifications were carried out in triplicate in a total volume of 20 μl containing 10 μl SYBR Green 2×Supermix (Applied Biosystems, USA), 5 μl of the 1:5 diluted cDNA, 1 μl each of forward and reverse primer and 3 μl PCR grade water. The qRT-PCR program was 50°C for 2 min, 95°C for 5 min, followed by 40 cycles of 94°C for 20s, 55°C for 30s, 72°C 30s. All analyses were based on the CT values of the PCR products. The CT was defined as the PCR cycle at which the fluorescence signal crossed a threshold line that was placed in the exponential phase of the amplification curve. Melting curve analysis of amplification products was performed at the end of each PCR reaction to confirm that only one PCR product was amplified and detected. After the PCR program, qRT-PCR data from three replicate samples were analyzed with a 7300 System SDS Software v1.3.0 (Applied Biosystems, USA) to determine the relative expression of each sample. To maintain consistency, the baseline was set automatically by the software. The comparative CT method was used to analyze the expression level of black tiger shrimp cyclin H. The CT for the target amplification of *Pm*cyclin H and the CT for the internal control β-actin were determined for each sample. Differences between the CT for the target and the internal control, called △CT, were calculated to normalize the differences in the amount of total nucleic acid added to each reaction and the efficiency of the RT-PCR. The blank group was used as the reference sample, called the calibrator. The △CT for each sample was subtracted from the △CT of the calibrator; the difference was called △△CT value. The expression level of black tiger shrimp cyclin H could be calculated by 2-△△CT, and the value stood for an n-fold difference relative to the calibrator. The average cycle threshold (CT) measurement for the three determinations were used in calculations of relative expression using β-actin as the internal control. The data obtained from RT-PCR analysis were subjected to one-way analysis of variance (one-way ANOVA) followed by an unpaired, two-tailed t-test. Differences were considered significant at $P < 0.05$.

Results

Cloning and sequence analysis of Pmcyclin H gene. One EST from the cDNA library of black tiger shrimp *Penaeus monodon* was homologous to the previously known cyclin H genes. Based on this EST, a 421 bp DNA fragments were amplified by 3′-RACE technique. A 1280 bp nucleotide sequence representing the complete cDNA sequence of *Pm*cyclin H was obtained by overlapping the fragments with this EST. The full-length cDNA sequence and the deduced amino acid sequence are shown in Fig. 1.

```
   1 TTCGGGAAGCGCAGGATTCAGCAAGGGAATGAAACCTGGAACAGAAATATCACAAACAAC 60
  61 AGAATGTATCTCAGTAGCACACAGTATCAGAATTGGACTTTCCGGGATGAACACGAAGTG 120
   1    M  Y  L  S  S  T  Q  Y  Q  N  W  T  F  R  D  E  H  E  V  19
 121 ATAAAGTTGCGATTTCAGGCCAATCATGACTTCATTGCAAAATTTGGGAGCAGCATGTCA 180
  20 I  K  L  R  F  Q  A  N  H  D  F  I  A  K  F  G  S  S  M  S  39
 181 CTGCAGGAGAAGATGCTGTTCTTCCTATCAGTTGAAGAGGAGCACATAATGGTACGCACT 240
  40 L  Q  E  K  M  L  F  F  L  S  V  E  E  E  H  I  M  V  R  T  59
 241 TATGAATATTCCTTGAGAGACTTTTGCAAAAAGTTTCGAGACCCCAGAGATGGAAGAATC 300
  60 Y  E  Y  S  L  R  D  F  C  K  K  F  R  D  P  R  D  G  R  I  79
 301 AGGATGCCTCCAGCAGTGACAACAACAGCACAACACTACTTTAAGAGATTTTATTTATTC 360
  80 R  M  P  P  A  V  T  T  T  A  Q  H  Y  F  K  R  F  Y  L  F  99
 361 AATTCTGTCATGGATTATCATCCCAAAGAAATTCTAGTGACCTGTGTATATCTTGCTTGT 420
 100 N  S  V  M  D  Y  H  P  K  E  I  L  V  T  C  V  Y  L  A  C  119
 421 AAAATTGAAGAATTTTATGTCACAATCAATGATTTTGTGCATAATGTAAGAGGAGATAAG 480
 120 K  I  E  E  F  Y  V  T  I  N  D  F  V  H  N  V  R  G  D  K  139
 481 AAGAAAGCTGCTGAGATTATTTTAAACAATGAACTGCAGCTAACACAAGAATTACAATTT 540
 140 K  K  A  A  E  I  I  L  N  N  E  L  Q  L  T  Q  E  L  Q  F  159
 541 CATCTCATTATTCACCAACCATTCCGCCCAGTAGAAGGCTTGCTCATTGACATTAAGACA 600
 160 H  L  I  I  H  Q  P  F  R  P  V  E  G  L  L  I  D  I  K  T  179
 601 AGGTTCCCACAGCTAAGAGATCCAGAAAGATTGCGACCCCATGTGGAAGAGTTCCTTGAA 660
 180 R  F  P  Q  L  R  D  P  E  R  L  R  P  H  V  E  E  F  L  E  199
 661 AGGGTAAACTTAACTGATGCAATTATTCTATATACTCCTGGTCAGATTGCTTTAGCTGCA 720
 200 R  V  N  L  T  D  A  I  I  L  Y  T  P  G  Q  I  A  L  A  A  219
 721 GTGACAACAGCTGTGAGCAGGTTAGGGGAAAACCTGGACCAGTATGTCACCGATATCCTC 780
 220 V  T  T  A  V  S  R  L  G  E  N  L  D  Q  Y  V  T  D  I  L  239
 781 TTCCCAAATGACCAAAGACCACACCTCAAAGTCCTTATAGATGCTGTGAGAAAAATCAAG 840
 240 F  P  N  D  Q  R  P  H  L  K  V  L  I  D  A  V  R  K  I  K  259
 841 AAGATGGTTAAGAATGCTGAACCTCCTGCCTCGGACACTGTGCGGCGTGAGATTGAAGGC 900
 260 K  M  V  K  N  A  E  P  P  A  S  D  T  V  R  R  E  I  E  G  279
 901 AAGTTAGAGAAGTGCAGAAATCAGCAAAACAATCCAAATTCATCCCAGTACCGGGCAAAC 960
 280 K  L  E  K  C  R  N  Q  Q  N  N  P  N  S  S  Q  Y  R  A  N  299
 961 TACTCAGAATGGGATGATGATGAGGTGATGATGGCTGCTTCCCCAATGCAGGAAGACACA 1020
 300 Y  S  E  W  D  D  D  E  V  M  M  A  A  S  P  M  Q  E  D  T  319
1021 GCTCTTGGAGTAGAGAGAATACGATCACCATCAAACTATTAACTTACTGTTTTTTTGTTT 1080
 320 A  L  G  V  E  R  I  R  S  P  S  N  Y  *                    332
1081 TTTTTTTCTCATTAATCATTTACTGGATTGTTTGTGTAAAATGTGAAAGTAATTTCTGAA 1140
1141 AGTTAAAGGAAAACCTTTGGTATTTTCTATCTTAAGTTGAGAGAGAGGGATGAAAAAATT 1200
1201 AAGATATATTTTGAAATGACATTTTTATTTTAAACTCCTAGTGAAAAGATAGAAAAAAAA 1260
1261 AAAAAAAAAAAAAAAAAAAA 1280
```

Fig .1 Full-length cDNA sequence and predicted amino acid sequence of *Pm*cyclin H. Numbers of the left and right of each row refer to nucleotide or amino acid position. The initiation codon (ATG) and the terminator codon (TAA) are boxed, the poly A signal sequence is italicized.

The full-length cDNA of *Pm*cyclin H consisted of a 5′-terminal un-translated region (5′UTR) of 63 bp, a 3′UTR of 218 bp with a 28bp poly (A) tail, and an open reading frame (ORF) of 999 bp. The ORF encoded a polypeptide of 332 amino acids with a predicted molecular weight of 39 kDa. Blastx analysis indicated that the deduced amino acid of *Pm*cyclin H shared high homology with other reported cyclin H, such as 80% positive to H-type cyclin from *Scylla paramamosain* (ACL81559.1), 54% to *Megachile rotundata* (XP003704261.1), and 40% to *Homo sapiens* (AAH22351.1).

SignalP 3.0 analysis revealed that cyclin H does not contain typical signal peptide sequence. SMART analysis showed that the amino acid region from 62 to 155 belonged to CYCLIN domain.

Phylogenetic analysis. A phylogenetic tree was reconstructed based on deduced amino acid sequence data of cyclin H (Fig. 2).

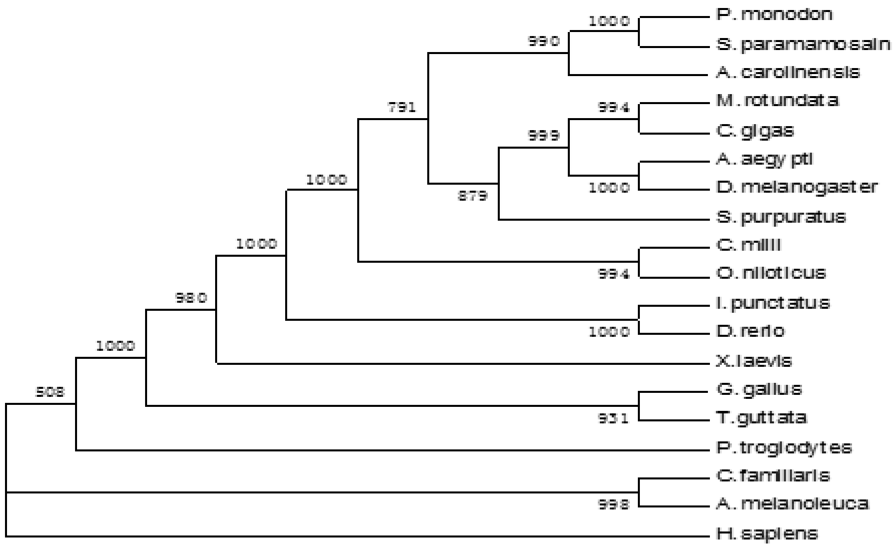

Fig.2 Phylogenetic tree of cyclin H sequences from different organisms using neighbor-join method. The deduced amino acid sequences of cyclin H proteins used in the phylogenetic analysis were from Genbank database under accession numbers ACL81559.1(*S. paramamosain*), XP003216334.1(*A. carolinensis*), XP003704261.1(*M. rotundata*), NP001013471.1(*D. rerio*), EKC20294.1(*C. gigas*), XP002186602.1(*T. guttata*), NP001081052.1(*X. laevis*), AAH22351.1(*H. sapiens*), AFK11589.1(*C. milii*), NP524207.1(*D. melanogaster*), XP003456105.1(*O. niloticus*), XP001656552.1(*A. aegypti*), XP787341.3(*S. purpuratus*), 424908.2(G. gallus), XP536300.3(C. familiaris), XP002913665.1（*A. melanoleuca*）, NP001187316.1(*I. punctatus*), BAK63165.1(*P. troglodytes*)

NJ tree from cyclin H consistently presented three well-defined clades with high statistical supports. *Pm*cyclin H was identified in most invertebrates' subgroup indicating that the identity of *Pm*cyclin H belonged to a new member of cyclin H family.

The three-dimensional structure of cyclin H about *P. monodon* and human were predicted by SWISS-MODEL (Fig.3).

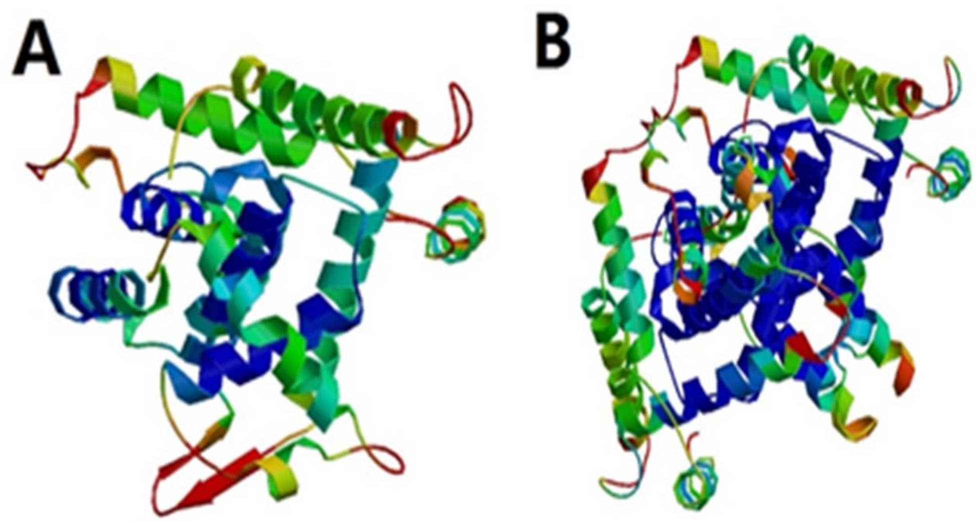

Fig.3. The three-dimensional ribbon structure of *Pm*cyclin H (A) compared with human cyclin H (B) which were predicted by Swiss-model and rasmol software.

The three-dimensional structure of *Pm*cyclin H has two characteristic α-helical domains with five helices for each one, the two α-helical domains were linked by a short hinge region giving the molecule its elongated shape (Fig.3-A); The core helix H3 (107-122aa) or H3′ (212-227aa) for each domain is flanked on one side by helices H1(50-70aa) and H2 (83-97aa) for the former or H1′ (167-180aa) and H2′ (188-202aa) for the latter. On the other sides, H4 (128-133aa) and H5 (142-156aa) or H4′ (233-238aa) and H5′ (249-263aa) were involved in each domain to form an interface with each one. A third domain, specific to cyclin H, was also identified in the *Pm*cyclin H, which consists of two long helices located from 17-36aa at the N-terminus and 271-286aa at C-terminus.

Tissues distribution of Pmcyclin H mRNA. Real time PCR was employed to quantify the expression of Pmcyclin H mRNA in different tissues of *P. monodon*. In healthy shrimp, the Pmcyclin H transcript was found to be constitutively expressed in a wide range of tissues with different expression levels. The expression of *Pm*cyclin H transcript was predominantly detectable in the tissue of ovary, to a lesser degree in the tissues of intestine, testis, stomach and heart, and almost indetectable in the liver, brain, and muscle tissues. (Fig. 4).

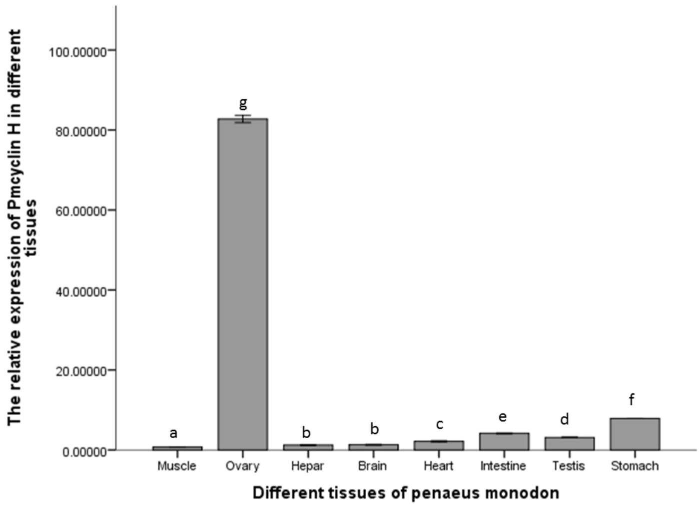

Fig.4. *Pm*Cyclin H mRNA expression pattern in different tissues.
Each symbol and vertical bar represented the mean ± S.E. (n = 3)

Pmcyclin H expression pattern in different stages of ovary development. Temporal effect of *Pm*cyclin H expression in ovarian development was investigated by real-time PCR (Fig. 5).

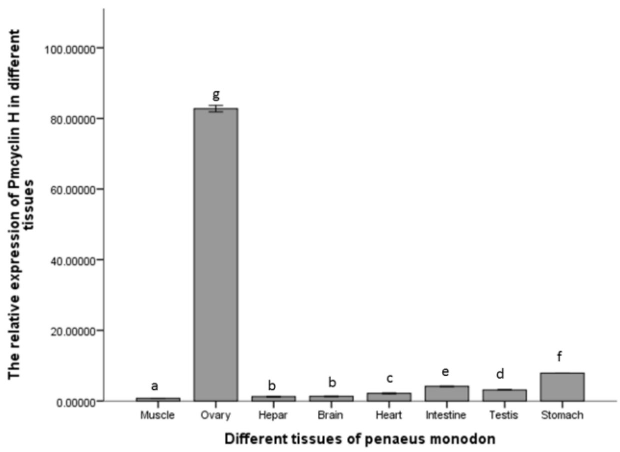

Fig.5. Time-course expression level of *Pm*cyclin H transcript during different developmental stages of ovary maturation.
Each symbol and vertical bar represented the mean ± S.E. (n = 3). I: primordial germ cell stage, II: chromatin nucleolus stage, III: perinucleolus stage, IV: yolky stage, V： cortical rod stage, VI: spent stage.

During the six developmental stages of the ovaries, the *Pm*cyclin H mRNA transcript was not different significantly from stage I to stage VI. One peak expression of *Pm*cyclin H was detected in stage II. The expression level detected in stage IV was similar to stage V. A second peak expression of *Pm*cyclin H was found in stage III, which was 1.33-fold increase compared to stage IV. Statistical analysis by SPSS17.0 of the six stages showed a significant difference in *Pm*cyclin H gene expression at stage II. However, no significant difference was observed in other stages.

Discussion

In this study, we identified and characterized cyclin H cDNA (named *Pm*cyclin H) in black tiger shrimp. The full-length cDNA is of 1280bp, including an ORF of 999 nucleotides encoding a polypeptide of 332 amino acids with an estimated molecular mass of about 39kDa. SignalP anlysis suggested that *Pm*cyclin H doesn't contain typical signal peptide sequences, and was not a secretory protein. *Pm*cyclin H has a cyclin domain ranging from Y62 to Q155.The high level of similarity of *Pm*cyclin H to cyclin H from other species suggests that *Pm*cyclin H should be a new member of the cyclin H family.

The three-dimensional structure of *Pm*cyclin H and human cyclin H were predicted by SWISS-MODEL. It was found that two characteristic ɑ-helical domains existed in Pmcyclin H and each ɑ-helical domain contained five helices, which are the typical three-dimensional structure characteristics of cyclin H (Jeffrey et al., 1995). Although the putative molecular modeling of *Pm*cyclin H had a different structure to that of human cyclin H, they are similar at the core of the structure. Phylogenetic analysis illustrated that *Pm*cyclin H was identified in most invertebrate subgroups. It indicated that *Pm*cyclin H had a closer relationship with invertebrate counterparts and perhaps played a similar role as invertebrate molecules. Cyclin H is well conserved from yeast to humans and shares significant homologies (Andersen et al., 1996; Andersen et al., 1997). Through homology analysis, it can be assume that cyclin H plays an important role for a vast majority of species.

Cyclin H is a very important regulatory factor in the cell cycle. Cyclin H together with CDK7 and MAT1 combined to form the complex of CAK, can regulate the activity of the CDK1, CDK2, CDK4 and CDK6 (Mäkelä et al., 1994; Graziano et al., 2005). The CDK1/CyclinB complex can promote the cell transition from S2 phage to M phage; the complex of CDK2/CyclinE, CDK4/CyclinD, and CDK6/CyclinD have the function of activating cell transition from G1 phage to S phage. The process of regulation is performed by dephosphorylation and phosphorylation of residues in the CDK (Morgan et al., 1995). Therefore, cyclin H, a cell cycle regulatory factor, plays a very important role in cell proliferation. Expression studies indicated that *Pm*cyclin H mRNA expression was tissue-specific. The level of *Pm*cyclin H expression in the ovaries was very strong, but was very weak in the tissues of muscle, liver and brain. This may be due to the characteristics of different organ growth. Cyclin H plays an important role in cell cycle regulation. Cyclin H is expressed in every cell cycle but the expression level may change (Fisher et al., 1994; Adamczewski et al., 1996). For example, distribution of cyclin H in zebrafish was found in the every tissue during early stages of development, and was limited to the anterior neural tube, brain, eyes, procreate tissues, liver and heart (Liu et al., 2007). *Pm*cyclin H mRNA expression also was different in the process of ovarian maturation. In the present study, *Pm*cyclin H displayed various expression levels during the ovarian maturation stages. In the second stage when the ovaries began to mature, gene expression was strongest. There was one peak expression of *Pm*cyclin H in stage III. The pattern of *Pm*cyclin H expression reflects the phase-specific regulatory function of the respective gene products. These results indicate that *Pm*cyclin H is involved in ovarian maturation stage transition and plays a role in the larval differentiation and development of the black tiger shrimp. Similar results were also observed in the early larval development of zebrafish (Liu et al., 2007).

The artificial breeding of parent shrimp restricts the development of the prawn breeding industry (Brady et al., 2012), as female shrimp are unable to reach ovarian maturity in captivity without eyestalk-ablation. However, the method of eyestalk-ablation is destructive and wild-caught broodstock is expensive (Treerattrakoo et al., 2011; Klinbunga et al., 2009). Results from the present study indicate that *Pm*cyclin H gene may be related to cell proliferation in the ovary and plays an important role in oocyte maturation. Through the regulation of *Pm*cyclin H expression, it may not only promote ovarian maturation of black tiger shrimp, but may also boost yields. the information gathered in this study may lay the foundation for further study of the mechanism of ovarian maturation.

Acknowledgements

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