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RESEARCH PAPER

Isolation and Expression Analysis of cAMP-Dependent Protein Kinase and Adenylyl Cyclase-Associated Protein 1-like cDNAs in the Giant Tiger Shrimp *Penaeus monodon*

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Abstract

Characterization of genes exhibiting differential expression profiles during ovarian development is important for understanding reproductive maturation of the giant tiger shrimp (*Penaeus monodon*). Here, the partial cDNAs of *P. monodon cAMP-dependent protein kinase, catalytic subunit 1* (*PmPkaC1*) and *adenylyl cyclase-associated protein 1-like* (*PmCap1-l*) were studied. They were more preferentially expressed in ovaries than testes of cultured juveniles and wild broodstock. *PmPkaC1* mRNA in ovaries of non-ablated broodstock was significantly increased during vitellogenesis ($P<0.05$). However, unilateral eyestalk ablation (the removal of one eyestalk) resulted in a significant reduction of its expression ($P<0.05$). *PmCap1-l* was not differentially expressed during ovarian development in wild non-ablated broodstock. It was up-regulated in mature ovaries following eyestalk ablation ($P<0.05$). The *PmCap1-l* transcript in each ovarian stage of the former was significantly lower than that of the latter ($P<0.05$). The levels of ovarian *PmPkaC1* and *vitellogenin 1* (*PmVtg1*) treated *in vitro* with 17α-20β-DHP (0.1, 1.0 and 10.0 µg/ml for 24 h) was not significantly different from the control ($P>0.05$). Nevertheless, the expression of *PmCap1-l* was increased in ovaries treated with 0.1 and 10 µg/ml 17α-20β-DHP at 24 hours post treatment (hpt, $P<0.05$).

Introduction

The giant tiger shrimp (*Peneaus monodon*) is one of the economically important cultured species. Farming of *P. monodon* in Thailand relies on wild-caught broodstock for supply of juveniles because of poor reproductive maturation of captive *P. monodon* females (Withyachumnarnkul et al., 1998; Preechaphol et al., 2007). Breeding of pond-reared *P. monodon* does not provide sufficient postlarvae with consistent quality required by the shrimp industry. Therefore, the use of genetically improved shrimp instead of wild broodstock is required for the sustainable aquaculture (Clifford and Preston, 2006; Coman et al., 2006). Nevertheless, low degrees of reproductive maturation of captive *P. monodon* has limited the ability to genetically improve this species by domestication and selective breeding programs (Withyachumnarnkul et al., 1998; Kenway et al., 2006; Preechaphol et al., 2007).

Ovarian maturation of penaeid shrimp is mainly regulated by gonad inhibiting hormone (GIH) producing from the X-organ/sinus gland located at the eyestalk

(Okumura, 2004; Meunpol et al., 2007). Eyestalk ablation is practically used for induction of ovarian maturation of penaeid shrimp by removing one eyestalk (Okumura, 2004; Ibara et al., 2007), but the technique leads to an eventual loss of spawners (Benzie, 1998). Therefore, predictable maturation and spawning of captive penaeid shrimp without the use of eyestalk ablation is a long-term goal for the shrimp industry (Quackenbush, 2001; Klinbunga et al., 2020).

Molecular mechanisms involving gonadal development of *P. monodon* have long been of interest (Benzie, 1998; Ibara et al., 2007; Preechaphol et al., 2007; Uengwetwanit et al., 2018). Characterization of differentially expressed genes (DEGs) during ovarian development could be applied for determining effects on stimulation of reproductive maturation of captive *P. monodon* by hormones, neurotransmitters and/or diets (Fingerman, M. & Nagabhushanam, R., 1992; Leelatanawit et al., 2004; Okumura, 2004; Preechaphol et al., 2010; Chimsung, 2014).

Progesterone and progestins are steroid hormones that functional contributed in gametogenesis (Mailer. & Krebs, 1977; Fingerman et al., 1993; Miura et al., 2006). In penaeid shrimp. progesterone promoted vitellogenesis and ovarian maturation (Kulkarni et al., 1979; Yano, 1985; Quackenbush, 2001). Likewise, 17α-hydroxyprogesterone played the same roles in *Marsupenaeus japonicus* (Yano, 1987). It also elevated spawning of *Metapenaeus ensis in vivo* (Yano, 1985). In *P. monodon*, progesterone showed prominent effects on stimulation of the final maturation while 17α-hydroxyprogesterone was effective in vitellogenesis of oocytes (Meunpol et al., 2007). Nevertheless, effects of vertebrate-like hormones (e.g. progesterone and its derivatives) on stimulation of ovarian development have not been well studied in penaeid shrimp at present.

Previous studies indicated that 17α,20β-dihydroxyprogesterone (17α-20β-DHP) is the maturation inducing substance (MIS) that promotes meiotic maturation in fish (Nagahama, 1997; Thomas, 2008). Therefore, molecular effects of 17α-20β-DHP on inducing expression of reproduction-related genes of economically important species like *P. monodon* should be studied. In this study, *P. monodon cAMP-dependent protein kinase, catalytic, subunit 1* (*PmPkaC1*) and *adenylyl cyclase-associated protein 1-like (PmCap1-l)* cDNAs were isolated and characterized. The expression levels of these genes during ovarian development in non-ablated wild broodstock were examined. Effects of unilateral eyestalk ablation on their expression levels were examined to verify whether this classical technique positively or negatively affected their expression. In addition, the short-term culture of ovarian explants was established for further examination on *in vitro* effects of 17α-20β-DHP to the expression levels of *PmPkaC1*, *PmCap1-l* and *vitellogenin 1* (*PmVtg1*).

Materials and Methods

Experimental Animals

For characterization of *PmPkaC1* and *PmCap1-l* cDNAs, wild female shrimp with vitellogenic ovaries (stage II, average body weight of 142.98±28.37 g) were collected alive from the Andaman Sea (west of peninsular Thailand) and transported back to the laboratory. Shrimp were acclimated for 3 days in the laboratory (28-30°C, 32 ppt seawater and natural light:dark period in 1000-liter fish tanks with aeration) before ovaries were collected and kept at -80°C for long storage.

For comparison of gene expression in males and females, juvenile shrimp (4-month-old) were obtained from a commercial farm in Chachengsao province (central Thailand, *N*=5 each of males and females, body weight of approximately 20 g). In addition, wild male and female adults (average body weight of 142.98±28.37 g and 212.77±43.33 g) were caught from the Andaman Sea and acclimated using the laboratory conditions (28-30°C and 32 ppt seawater under the natural daylight in 1000-liter fish tanks with aeration) for 3 days (*N*=5 for each sex). Ovaries and various tissues of wild females and testes of males were dissected out and subjected to tissue expression analysis.

For evaluation effects of eyestalk ablation, wild female shrimp were collected from the Andaman Sea and acclimated in a commercial farm (32 ppt salinity at 28-30°C under the dark condition with aeration in 10-ton tanks, *N*=40 each for non-ablated and ablation groups; average body weight of 217.07±47.10 g and 209.97±39.45 g) for 7 days. Ovaries of non-ablated shrimp were externally examined before dissected out. The ovarian developmental stages of wild *P. monodon* were classified according to gonadosomatic indices (GSI, ovarian weight/body weight x 100): <2, 2-4, >4-6 and >6% for previtellogenetic (stage I, *N*=10), vitellogenetic (stage II, *N*=5), late vitellogenic (stage III, *N*=7) and mature (stage IV, *N*=9) stages. Ovaries of non-ablated post-spawning broodstock were immediately collected and regarded as stage V (*N*=6) (Sittikankaew et al., 2010).

Moreover, acclimated shrimp (*N*=40, average body weight of 209.97±39.45 g) with previtellogenic ovaries were subjected to unilateral eyestalk ablation. One eyestalk of each female shrimp was excised using sterile scissors. The ovarian stages of ablated *P. monodon* were daily examined externally and shrimp ovaries were collected at 2 – 7 days after eyestalk ablation when they reached desired stages (I, II, III and IV; *N*=10, 5, 10 and 9, respectively).

For analysis of gene expression following *in vitro* treatment of 17α-20β-DHP, female adults were collected from the Andaman Sea (Table 1), transported back to the laboratory and acclimated as described above for 3 days (*N*=6) before subjected to the experiment (see below).

Preparation of Total RNA and First-Strand cDNA Synthesis

Total RNA was extracted from *P. monodon* tissues using TRI Reagent (Molecular Research Center). The obtained total RNA was treated with DNase I (0.5 U/µg total RNA at 37ºC for 30 min) to eliminate possible genomic DNA contamination. The resulting total RNA (1.5 µg) was subjected to the synthesis of first-strand cDNA using an Improm-II^{TM} Reverse Transcription System (Promega).

Amplification of the Partial PmPkaC1 Transcript Using Degenerate Primers

Nucleotide sequences of *cAMP-dependent protein kinase, catalytic, beta a-like* from various species were retrieved from GenBank (http://ncbi.nlm.nih.gov) and multiple-aligned using Clustal W (Thompson et al., 1994). Degenerate primers (DG-PmPkaC1-F1, DG-PmPkaC1-R1 and DG-PmPkaC1-F2, Table 2) were designed. RT-PCR was initially carried out using primers DG-PmPkaC1-F1/R1. The amplification product was

Table 1. Primers and primer sequences used in this study

Primers	Sequence
Degenerate RT-PCR	
DG-PmPkaC1-F1	5´-GA(A/G)CA(T/C)AA(A/G)GA(A/G)CA(T/C)AA(A/G)GA(T/C) TA(T/C)TT(T/C)-3´
DG-PmPkaC1-R1	5´-AT(T/C/A)TA(T/C)GA(A/G)AA(A/G)AT(T/C/A)GT(N)AG(T/C) GG(N)AA(A/G)-3´
DG-PmPkaC1-F2	5´-AA(A/G)AG(T/C)GG(N)AG(A/G)TT(TC)TC(N)GA(A/G)-3´
RACE-PCR	
5´RACE-PkaC1	5´-CAGCGGCCATTTCATACACCAGCAC-3´
3´RACE-PkaC1	5´-GGGTTACAACAAGGCTGTGGACTGGT-3´
5´RACE-Cap1	5´-CAATGTCAACCTGAAGGCTGCTCC-3´
3´RACE-Cap1	5´-CCTGGTGTCTTCTGCGGAGGTTGT-3´
nested-5´RACE-Cap1	5´ ACTTGCCTACGATGA CCTCCTCTCG-3´
RT-PCR	
RT-PmPkaC1-F	5´-TCACGCTGGCTACAGAGTGGAGG-3´
RT-PmPkaC1-R	5´-AACTCCTACA AAGAACACGGCGT-3´
RT-PmCap1-F	5´-TGGACGCC ACAGACAGTTAGAG-3´
RT-PmCap1-R	5´-TGGCCATCACCTGGACCTT-3´
RT-EF-1α_{500}-F	5´-ATGGTTGTCAACTTTGCCCC-3´
RT-EF-1α_{500}-R	5´-TTGAACTCCTTGATCACACC-3´
qRT-PCR	
qRT-PmPkaC1-F	5´-TCACGCTGGCTACAGAGTGGAGG-3´
qRT-PmPkaC1-R	5´-ACGCCGTGTTCTTTGTAGGAGTT-3´
qRT-PmCap1-F	5´-GTCCCACTGGTGCCAAAAGC-3´
qRT-PmCap1-R	5´- CTGGTGGAAGACGAGGTGCG -3
qRT-PmVtg-F	5´- AGGCATCACAGTAACTGAGACCGAT -3´
qRT-PmVtg-R	5´- CAGGTGTTGGGTAACGTTCTTGAC -3´
qRT-EF-1α_{214}-F	5'-GTCTTCCCCTTCAGGACGTC-3'
qRT-EF-1α_{214}-R	5'-CTTTACAGACACGTTCTTCACGTTG-3'

Table 2 Primers and primer sequences used in this study

Primers	Sequence
Degenerate RT-PCR	
DG-PmPkaC1-F1	5´-GA(A/G)CA(T/C)AA(A/G)GA(A/G)CA(T/C)AA(A/G)GA(T/C) TA(T/C)TT(T/C)-3´
DG-PmPkaC1-R1	5´-AT(T/C/A)TA(T/C)GA(A/G)AA(A/G)AT(T/C/A)GT(N)AG(T/C) GG(N)AA(A/G)-3´
DG-PmPkaC1-F2	5´-AA(A/G)AG(T/C)GG(N)AG(A/G)TT(TC)TC(N)GA(A/G)-3´
RACE-PCR	
5´RACE-PkaC1	5´-CAGCGGCCATTTCATACACCAGCAC-3´
3´RACE-PkaC1	5´-GGGTTACAACAAGGCTGTGGACTGGT-3´
5´RACE-Cap1	5´-CAATGTCAACCTGAAGGCTGCTCC-3´
3´RACE-Cap1	5´-CCTGGTGTCTTCTGCGGAGGTTGT-3´
nested-5´RACE-Cap1	5´ ACTTGCCTACGATGA CCTCCTCTCG-3´
RT-PCR	
RT-PmPkaC1-F	5´-TCACGCTGGCTACAGAGTGGAGG-3´
RT-PmPkaC1-R	5´-AACTCCTACA AAGAACACGGCGT-3´
RT-PmCap1-F	5´-TGGACGCC ACAGACAGTTAGAG-3´
RT-PmCap1-R	5´-TGGCCATCACCTGGACCTT-3´
RT-EF-1α_{500}-F	5´-ATGGTTGTCAACTTTGCCCC-3´
RT-EF-1α_{500}-R	5´-TTGAACTCCTTGATCACACC-3´
qRT-PCR	
qRT-PmPkaC1-F	5´-TCACGCTGGCTACAGAGTGGAGG-3´
qRT-PmPkaC1-R	5´-ACGCCGTGTTCTTTGTAGGAGTT-3´
qRT-PmCap1-F	5´-GTCCCACTGGTGCCAAAAGC-3´
qRT-PmCap1-R	5´- CTGGTGGAAGACGAGGTGCG -3
qRT-PmVtg-F	5´- AGGCATCACAGTAACTGAGACCGAT -3´
qRT-PmVtg-R	5´- CAGGTGTTGGGTAACGTTCTTGAC -3´
qRT-EF-1α_{214}-F	5'-GTCTTCCCCTTCAGGACGTC-3'
qRT-EF-1α_{214}-R	5'-CTTTACAGACACGTTCTTCACGTTG-3'

diluted 50-fold and used as the template for reamplification using primers DG-PmPkaC1-F2/R1. The amplification product was size-fractionated and eluted from the agarose gel. The eluted PCR product was ligated to pGEM-T-Easy vector (Promega) and transformed into *E. coli* JM109 (Sambrook and Russell, 2001). Recombinant plasmid DNA was extracted and sequenced for both directions.

Rapid Amplification of cDNA End-Polymerase Chain Reaction (RACE-PCR)

Non-treated total RNA was further purified using a QuickPrep Micro mRNA Purification Kit (GE Healthcare). 5′- and 3′RACE-PCR template was separately synthesized using a SMART RACE cDNA Amplification Kit (BD Bioscience Clontech). Gene-specific primers were designed (5′- and 3′RACE-PkaC1 and 5′-, 3′RACE-Cap1 and nested-5′RACE-Cap1, Table 2). RACE-PCR was carried out. The amplified fragments were cloned and sequenced. After sequence assembly, similarity search was performed using Blast*X* (Altschul et al., 1990). Functional domains of the deduced PmPkaC1 and PmCap1 proteins were predicted using SMART (http://smart.embl-heidelberg.de).

Phylogenetic Analysis

The deduced amino acid sequence of *cAMP-dependent protein kinase catalytic subunit 1* gene of *Procambarus clarkii* (QIA97602.1), *Litopenaeus vannamei* (XP_027236571.1, XP_027236572.1 and XP_027236573.1), *Marsupenaeus japonicus* (XP_042877329.1 and XP_042877330.1), *Daphnia magna* (XP_032787603.1), *Octopus bimaculoides* (XP_014777151.1), *Mytilus galloprovincialis* (VDI64276.1), *Sepia pharaonis* (CAE1178605.1), *Armadillidium nasatum* (KAB7498376.1), *Thrips palmi* (XP_034253029.1), *Melanaphis sacchari* (XP_025202275.1), *Pecten maximus* (XP_033737661.1), *Mizuhopecten yessoensis* (XP_021365871.1), *Pollicipes pollicipes* (XP_037086825.1 and XP_037069978.1), *Tribolium castaneum* (XP_008199609.1), *Crassostrea gigas* (XP_011439334.1) and *Aedes aegypti* (XP_001652671.1) were retrieved and phylogenetically analyzed with that of *P. monodon*.

In addition, the deduced amino acid sequences of *adenylyl cyclase-associated protein 1-like* gene of *Litopenaeus vannamei* (XP_027212254.1, XP_027212253.1, XP_027212258.1, XP_027212257.1, XP_027212255.1 and XP_027212256.1), *Marsupenaeus japonicus* (XP_042888689.1, XP_042888692.1, XP_042888687.1, XP_042888688.1, XP_042888691.1 and XP_042888690.1), *Homarus americanus* (XP_042222726.1, XP_042222725.1, XP_042222728.1, XP_042222729.1 and XP_042222724.1), *Procambarus clarkii* (XP_045612543.1, XP_045612544.1, XP_045612547.1, XP_042222723.1, XP_045612548.1, XP_045612545.1 and XP_045612546.1), *Daphnia magna* (KZS09361.1), *Poecilia formosa* (XP_007576532.1, XP_016518702.1 and XP_016518701.1) and *Poecilia latipinna* (XP_014909930.1 and XP_014909929.1) were retrieved from GenBank (http://ncbi.nlm.nih.gov) and compared with that of *P. monodon* in this study.

Multiple alignments were carried out with ClustalW (Thompson et al., 1994). A bootstrapped neighbor-joining tree (Saitou and Nei, 1987) was constructed using MEGA 7.0 (Kumar et al. 2016).

RT-PCR and Tissue Distribution Analysis

Expression of *PmPkaC1* (primers RT-PmPkaC1-F/R) and *PmCap1-l* (primers RT-PmCap1-F/R) in ovaries and testes of *P. monodon* juveniles and broodstock (*N*=5 for each group) was analyzed by a conventional RT-PCR (Sittikankaew et al., 2010). $EF\text{-}1\alpha_{500}$ (primers RT-EF-$1\alpha_{500}$-F/R) was included as the positive control. The amplicon was analyzed by agarose gel electrophoresis (Sambrook and Russell, 2001). Expression of *PmPkaC1* and *PmCap1-l* mRNAs in various tissues of wild females was assessed in the same manner.

Quantitative real-time PCR (qRT-PCR)

Recombinant plasmids of $PmPkaC1_{171}$, $PmCap1\text{-}l_{133}$, $PmVtg1_{134}$ and $EF\text{-}1\alpha_{214}$ were constructed. Standard curves covering 10^3-10^8 copies of the target ($PmPkaC1_{171}$:, $PmCap1\text{-}l_{133}$ and $PmVtg1_{134}$) and reference mRNAs ($EF\text{-}1\alpha_{214}$) were generated. The target and $EF\text{-}1\alpha_{214}$ mRNAs in ovaries of each shrimp were separately amplified in a 10 µl reaction volume containing 5 µl of 2x LightCycler 480 SYBR Green I Master (Roche), 100 ($PmPKACB_{171}$, $PmCAP1_{133}$ and $PmVtg1_{134}$) or 5 ($EF\text{-}1\alpha_{214}$) ng the first strand cDNA template and 0.2 ($PmPKACB_{171}$, $PmVtg1_{134}$ and $EF\text{-}1\alpha_{214}$) or 0.3 µM ($PmCAP1_{133}$) each primer. The thermal profiles for quantitative real-time PCR were 95°C for 10 min followed by 40 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 30 s. The analysis of crossing points (Cp) of standard curves and experimental samples was performed using the second derivative maximum method of the LightCycler software. The quantification of $PmPKACB_{171}$, $PmCAP1_{133}$, $PmVtg1_{134}$ and $EF\text{-}1\alpha_{214}$ mRNA in each sample well was evaluated by reference to the relevant standard curve. The relative expression levels (copy number of each target transcript/that of $EF\text{-}1\alpha_{214}$) between shrimp having different stages of ovarian development (or different time intervals following 17α-20β-DHP treatment) were statistically tested using one way analysis of variance (ANOVA) and Duncan's new multiple range test (P<0.05).

Ovarian Organ Culture and 17α-20β-DHP Treatment

Ovaries were dissected out from each shrimp (average body weight of 239.80 ± 22.10 g with GSI=1.45

– 2.00%, *N*=6) and rinsed three times with sterilized saline solution (2.8% NaCl supplemented with penicillin G and streptomycin (1,000 μg/ml each) and four times with M199 containing 5% FBS and 100 U/ml each of penicillin and streptomycin. Only the middle lobes of ovaries were used and cut into 3-5 mm in size in the culture medium (M199 with 10% FBS, 100 U/ml of each antibiotic, 2 mM L-glutamine and 10 mM HEPES, pH 7.3). A stock solution of 100 μg/ml of 17α-20β-DHP (Sigma) was prepared by dissolved in propylene glycol (PPG) : absolute ethanol (1:1 v/v). Four ovarian pieces were placed in 1 ml of M199 containing either 17α-20β-DHP (0.1, 1.0 and 10.0 μg/ml treatment) or the vehicle control (PPG: absolute ethanol 1:1). A piece of ovarian tissue from each individual was collected after incubated at 28 °C for 0, 1, 3, 6, 12 and 24 hours post treatment (hpt), placed in an Eppendorf tube containing 1 ml of the RNAlater™ stabilization solution and kept at -20°C. The first-strand cDNA was prepared and qRT-PCR was performed as described previously.

Results

Isolation and Characterization of *PmPkaC1* and *PmCap1-l* cDNAs

Degenerate primers designed from various cDNA sequences of *PKACB* generated the amplification product of 215 bp. This fragment was cloned and sequenced and it was significantly matched *cAMP-dependent protein kinase, catalytic, beta a-like* of *Saccoglossus kowalevskii* (*E*-value=8e-35) (data not shown). RACE-PCR was further carried out and the positive amplification bands from 5′- and 3′RACE-PCR were cloned and sequenced.

The partial cDNA sequence of *P. monodon cAMP-dependent protein kinase, catalytic subunit 1* (*PmPkaC1*) was successfully isolated and it was 2211 bp composing the partial ORF of 1053 bp deduced to 350 amino acids with the 3′ untranslated regions (UTRs) of 1158 bp (Figure 1). Its significantly matched *cAMP-dependent*

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ACGATGGGAAATGCGGCCACAGCCAAGAAAGGCGACCCAGCAGAGAATGTCAAAGAGTTC 60
T  M  G  N  A  A  T  A  K  K  G  D  P  A  E  N  V  K  E  F   20
CTCGAGAAGGCGAAAGAAGACTTCGAAGAAAAATGGAAAACTCCTACAAAGAACACGGCG 120
L  E  K  A  K  E  D  F  E  E  K  W  K  T  P  T  K  N  T  A   40
TGCTTGGACGACTTCGGGCGCATCAAGACCCTGGGCACCGGCTCCTTCGGGCGGGTGATG 180
C  L  D  D  F  G  R  I  K  T  L  G  T  G  S  F  G  R  V  M   60
CTGGTGCAGCACAAGTCCACCAAGGAATACTACGCCATGAAGATACTCGACAAACAGAAG 240
L  V  Q  H  K  S  T  K  E  Y  Y  A  M  K  I  L  D  K  Q  K   80
GTGGTTAAGTTGAAACAAGTGGAACACACGCTCAACGAGAAGCGAATATTGCAAGCCATC 300
V  V  K  L  K  Q  V  E  H  T  L  N  E  K  R  I  L  Q  A  I   100
AGTTTCCCGTTCCTCGTCAGCCTTGAATTCCACTTCAAGGATAACACAAATTTATACATG 360
S  F  P  F  L  V  S  L  E  F  H  F  K  D  N  T  N  L  Y  M   120
GTGCTAGAATATGTCCCCGGCGGCGAGATGTTCTCTCACTTGCGACGTCTAGGAAGATTT 420
V  L  E  Y  V  P  G  G  E  M  F  S  H  L  R  R  L  G  R  F   140
AGTGAACCACATTCGCGTTTCTATGCGGCCCAGATCGTGCTAGCGTTCGAATACCTTCAC 480
S  E  P  H  S  R  F  Y  A  A  Q  I  V  L  A  F  E  Y  L  H   160
TACCTAGATCTCATATACAGAGATCTTAAGCCAGAGAATCTACTTATAGACAGTCTAGGG 540
Y  L  D  L  I  Y  R  D  L  K  P  E  N  L  L  I  D  S  L  G   180
TACCTTAAGGTGACAGACTTCGGGTTCGCGAAGCGGGTGAAGGGGCGAACGTGGACGCTG 600
Y  L  K  V  T  D  F  G  F  A  K  R  V  K  G  R  T  W  T  L   200
TGTGGCACGCCGGAATACCTCGCTCCGGAGATCATCCTCTCCAAGGGTTACAACAAGGCT 660
C  G  T  P  E  Y  L  A  P  E  I  I  L  S  K  G  Y  N  K  A   220
GTGGACTGGTGGGCGCTGGGTGTGCTGGTGTATGAAATGGCCGCTGGATACCCTCCCTTC 720
V  D  W  W  A  L  G  V  L  V  Y  E  M  A  A  G  Y  P  P  F   240
TTTGCTGACCAGCCTATACAGATTTACGAGAAGATTGTATCTGGAAAGGTGAGGTTCCCT 780
F  A  D  Q  P  I  Q  I  Y  E  K  I  V  S  G  K  V  R  F  P   260
GGCCACTTCTCCTCAGACCTGAAGGATCTCTTGAGGAACCTGCTGCAGGTGGATCTCACC 840
G  H  F  S  S  D  L  K  D  L  L  R  N  L  L  Q  V  D  L  T   280
AAGCGCTACGGCAACTTGAAGAATGCCGTCAACGATATTAAGAATCACAAGTGGTTCGCA 900
K  R  Y  G  N  L  K  N  A  V  N  D  I  K  N  H  K  W  F  A   300
TCAACAGACTGGATTGCAGTATACCAGAAGAAGGTTGAAGCTCCATTTATTCCCAAGTGC 960
S  T  D  W  I  A  V  Y  Q  K  K  V  E  A  P  F  I  P  K  C   320
AAAGGCCCAGGAGACACCAGCAACTTTGACGACTATGAGGAGGAAGCTCTTCGTATTTCT 1020
K  G  P  G  D  T  S  N  F  D  D  Y  E  E  E  A  L  R  I  S   340
TCTACGAAAAAAATGTGCCAAGGAGTTTGCTGAGTTCTAGAGGCCTTTTGCAGTGTCTAG 1080
S  T  K  K  M  C  Q  G  V  C  *                              350
AGGCTAAGCTGACTCCCTGTGTCTGTGTACAAGTTTAGTACTCCCCTCCAGAGACCCCAG 1140
TGCATGTGCGTTGCTGTATACATGAGGCTGTGTGCGCACCATTGGTGCCATGCAGTCAGG 1200
CCTCAGGCAAGCAGTGGGCTAAGAAATAATGCAGCAGGTGCTCAGTTGTGGGAGAAGCTG 1260
TTTACTTGGTGGCTCACACACATGCAGACATTGCCAGAGAGCCCAGGCTGTGATGGCAGA 1320
TGTGCTACAGATCAGAATGGTGATGTGGCATAGGAAGCCGCCAAGGTCAGGATTCTTCAG 1380
TGTCACTTGAAATCAGCTTGATAAGGCAATATGAACAAACAGCTTAATGTTTTTATTATT 1440
ATTATGATCTTTCCCATTATTACCATTGTCGTTTTTATTTTCATCATTAAGGTGAAAGAA 1500
TACCTCTGTGTCAATCCGAAGAAATATAGCCACTTGATTTCCTAGAAAGCAAGTTGACGT 1560
CAAGTAACATTCAAGATCCAGTTTTGCCTTTTGTGTTGATGTGTGCCACCTCAGGAAGAT 1620
TGCCAGACAGAGGCTGTCACGTGATGGCGGGTAAATGAATGGCTTNCATTGTGGTCATGG 1680
GTGCAAGTGCCATTTTTAGCAAATAAGGTGGCGTCTGTGAGGAAGTGTGCCGCCNCAAGC 1740
CANCCACTTGGGNTNTGGGAGNTAACCTANTCATGTGGTGAGTGGGGGGTTGGTGGGCTT 1800
GTCGGGAAGAACACCANTAAAAGAANGGAGACATACAGATGGACTGGAGGTTGCACNCAT 1860
GGTGAACATTTACTGCAGTGAGCTGAATATCGGTNGGTGCTTTGGAGTAGCATTCAGAGT 1920
CCCCCCCTCCCCCTGTNTACTNTCGGTAGTGTNGGGGACGTACCAGGANCCTTCAAGGAT 1980
TNCCGTTCATGATNTTTCAGTGCAGGANGTATGCAACACCTTCAGCTTGAGTANAAAAGT 2040
AGGAGTGATAATTTTTTCNTCAGACTTTTTTTTTTTYTTNTTTTTTTNTTNTCCATAACA 2100
CATAACAATTAGTATACAAATAGTGATCATNTAATAATGTTTCCTACAGNGGCAAAATGA 2160
TGCACAACACTGNGAAATTANTCAAAAAAAAAAAAAAAAAAAAAAAAAAAA            2211
```

Figure 1. The partial cDNA sequences of *PmPkaC1*. Start and stop codons are illustrated in boldface and underlined. S_TKc and S_TK X domains are highlighted.

protein kinase catalytic subunit 1 (*PkaC1*) of *Procambarus clarkii* (accession no. QIA97602.1, *E*-value=0.0). The predicted Serine/Threonine protein kinases, catalytic domain (S_TKc, *E*-value=2.98e-104) and Ser/Thr-type protein kinase domain (S_TK X, *E*-value=8.61e-09) were located at amino acid positions 45-297 and 300-350 of the deduced PmPKAC1 protein.

In addition, the partial *PmCap1-l* cDNA was isolated by RACE-PCR and it 1708 bp containing the partial ORF of 1647 bp (549 amino acids) with the 3´UTR of 61 bp (Figure 2). Its closest similarity was *adenylyl cyclase-associated protein 1-like* (*Cap1-l*) of the Pacific white shrimp *Litopenaeus vannamei* (accession no. XP_027212254.1, *E*-value= 0.0). The deduced *PmCAP1-l* protein contained the cyclase-associated proteins N terminal (CAP_N) domain at amino acid positions 80-341 (*E*-value=1e-92) and CAPs and X-linked retinitis pigmentosa 2 gene product (CARP) at amino acid positions 429-466 (*E*-value=1.40e-10) and 467-504 (*E*-value=1.28e-09).

Phylogenetic Analysis of *PmPkaC1* and *PmCap1-l*

A phylogenetic analysis indicated that genes encoding cAMP-dependent protein kinase catalytic subunit 1 protein of *Procambarus clakii*, *Litopenaeus vannamei* (isoforms X1, X2 and X3) and *PmPkaC1* (this study) was clearly differentiated from insects and molluscs (Figure 3). Three different isoforms of deduced PkaC1 proteins of *L. vannamei* were phylogenetically clustered with *PmPkaC1* (bootstrapping value=84%).

Multiple isoforms of *adenylyl cyclase-associated protein 1-like* genes were found in various species. A bootstrapped neighbor-joining tree of deduced amino acids of this gene revealed closed relationships between crustacean species (shrimp, lobster and crayfish). *PmCap1-l* clustered with *L. vannamei Cap1-l* isoform X6 (bootstrapping value=100%) (Figure 4).

```
ACGCGGGGGAGTATCTGGTCTCTGTTATACTCTCTCTATAGTCAGATCCAGGCCGATAGT 60
  R  G  G  V  S  G  L  C  Y  T  L  S  I  V  R  S  R  P  I  V 20
CGCACACTGCAAGGTCGTCCATTGCGGGTGCGTGAATTCGATACTAATACGACTCACTAT 120
  A  H  C  K  V  V  H  C  G  C  V  N  S  I  L  I  R  L  T  I 40
AGGGCTAGCAGTGGGTATCTTGACACAGATTACGCAGAGAGTTGATAGCTTTGTAGATGC 180
  G  L  A  V  G  I  L  T  Q  I  T  Q  R  V  D  S  F  V  D  A 60
GCGGTGGCGGGTGTGTCTAGTGGTCGGCTCTTACAACCGGTGTGCAAGTGTAGGCAGAGT 240
  R  W  R  V  C  L  V  V  G  S  Y  N  R  C  A  S  V  G  R  V 80
TAGTATAGAGTTTCTAGGTAAAGTTTGGGACACGGAAGAAACAATGTCAGAGGATTGCTC 300
  S  I  E  F  L  G  K  V  W  D  T  E  E  T  M  S  E  D  C  S 100
AGTGCCATTCGTACTTGCCTACGATGACCTCCTCTCAGGCCCCTTCAAGGCCTTCCTGGA 360
  V  P  F  V  L  A  Y  D  D  L  L  S  G  P  F  K  A  F  L  D 120
CACGAGTAACCAGATTGGGGGAGATGTCGCTGCAATTGCAAAGATGGTGCAGGAAACATT 420
  T  S  N  Q  I  G  G  D  V  A  A  I  A  K  M  V  Q  E  T  F 140
CATCAATCAGCGAGCTTTCTTGGTCATGGCTTCAAAGAGCCAGCGACCCCTTGACAGTGA 480
  I  N  Q  R  A  F  L  V  M  A  S  K  S  Q  R  P  L  D  S  E 160
ACTGCCTCAGGTACTAGAGCCAACTGGCAAGAAGGTCCAGGAAGTGATTGCATTTAGGGA 540
  L  P  Q  V  L  E  P  T  G  K  K  V  Q  E  V  I  A  F  R  E 180
ATCTAAACGACAGTCGCCATTCTTCAATCACCTGTCTGCAGTGTCCGAGTCTATTCCTGG 600
  S  K  R  Q  S  P  F  F  N  H  L  S  A  V  S  E  S  I  P  G 200
CCTATCATGGGTGGCTGTTTCTCCAGCACCAGCACCTTATGTTAAGGAAATGGGAGACTC 660
  L  S  W  V  A  V  S  P  A  P  A  P  Y  V  K  E  M  G  D  S 220
TGCAGTATTCTATACTAACAGGGTCCTCAAGGAATACAAGGACAAGGACAAAGTTCACGT 720
  A  V  F  Y  T  N  R  V  L  K  E  Y  K  D  K  D  K  V  H  V 240
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  E  W  V  Q  H  W  N  N  V  F  K  E  L  Q  Q  Y  V  K  T  H 260
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  A  P  A  N  C  G  V  P  P  P  P  P  P  G  I  P  P  P  P  P 300
AATGGTCCCACTGGTGCCAAAAGCCGTGGCCGCGGAGGATGACGGCCGAGCTGCACTCTT 960
  M  V  P  L  V  P  K  A  V  A  A  E  D  D  G  R  A  A  L  F 320
CGCCTCTATCAATAAGGGAACAGATATCACATCCGGGCTCAAGAAGGTGGACCGCAACGC 1020
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ACCTCGTCTTCCACCAGGGGTGTGGACGCCACAGACAGTTAGAGATGAACAGAAAACTCA 1080
  P  R  L  P  P  G  V  W  T  P  Q  T  V  R  D  E  Q  K  T  H 360
CAAAAACCCCAGTCTGCGTGAGGGACCAAAACCATTCGTCAAGCAGACCGAGACTACCCC 1140
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TGTCCGTGCACCCACTCGCGAGTCACCGGAACGCCCTCCCAAGTTTGCTCTTGAAGGGAA 1200
  V  R  A  P  T  R  E  S  P  E  R  P  P  K  F  A  L  E  G  K 400
GAAGTGGTTTGTGGAATACCAGAAAGACAAACCAAGTCTTGTGGTCGATAATGCTAGGAT 1260
  K  W  F  V  E  Y  Q  K  D  K  P  S  L  V  V  D  N  A  R  M 420
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  N  S  V  I  L  D  S  C  K  K  S  A  V  V  F  D  N  L  V  S 460
TTCTGCGGAGGTTGTCAATTGTCAGTCGTGCAAGGTCCAGGTGATGGGCATTATGCCAAC 1440
  S  A  E  V  V  N  C  Q  S  C  K  V  Q  V  M  G  I  M  P  T 480
CATCACGATTGAGAAGACCGATGGTTGCCATGTATACCTGAGCAAAGAGTCTCTGAACAC 1500
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AGAGATCATTTCAGCCAAGTCCTCAGAAATGAACATCCTCATCCCCAAAGAGGATGGAGA 1560
  E  I  I  S  A  K  S  S  E  M  N  I  L  I  P  K  E  D  G  E 520
GTTTGTAGAGTGTCCTGTTCCTGAGCAGTTCAAGACGGTGATCAAGGGCCATTCCCTGGT 1620
  F  V  E  C  P  V  P  E  Q  F  K  T  V  I  K  G  H  S  L  V 540
CACTACATGTACCGAAAAGGCTGGTTAGAATCGGTAAAACAATGCTCATGTAGAAGTAAA 1680
  T  T  C  T  E  K  A  G  *                                  548
AAAAAAAAAAAAAAAAAAAAAAAA                                     1704
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Figure 2. The partial cDNA sequences of *PmCap1-l*. Stop codon is boldfaced and underlined. The CAP and CARP domains are highlighted.

Expression of *PmPkaC1* and *PmCap1-l* in Various Tissues of *P. monodon*

A greater expression level of *PmPkaC1* than testes was found in both juveniles and wild broodstock of *P. monodon* (Figure 5). In female adults, *PmPkaC1* was highly expressed in thoracic ganglion and moderately expressed in intestine and ovaries. Low expression was observed in the remaining tissues (antennal gland, hemocytes, gills, subcuticular epithelium, heart, lymphoid organ, hepatopancreas, stomach, eyestalk and pleopod) (Figure 6).

Similarly, a preferential expression of *PmCap1-l* in ovaries than testes of juveniles and adults (*N*=5 for each) of *P. monodon* was also observed (Figure 7). Abundant expression of *PmCap1-l* was found in ovaries, hemocytes and lymphoid organ. Limited expression was observed in other tissues (gills, heart, hepatopancreas, stomach, intestine, thoracic ganglion, eyestalk and pleopod) of female broodstock and testes of male broodstock (Figure 8).

Expression Levels of *PmPkaC1* and *PmCap1-l* in Different Stages of Ovaries of Non-ablated and Ablated *P. monodon* Broodstock

In non-ablated shrimp, the expression level of *PmPkaC1* in premature ovaries of juveniles and previtellogenic ovaries of broodstock was not significantly different (P>0.05). Its expression level was significantly increased in vitellogenic (stage II) and late vitellogenic (stage III) ovaries (P<0.05) before slightly reduced in mature (stage IV) ovaries and those of post-spawning shrimp (stage V) (P>0.05). *PmPkaC1* was comparably expressed in ovaries of eyestalk-ablated broodstock. Interestingly, eyestalk ablation resulted in a reduction of its expression in stages II, III and IV but results were statistically significant in vitellogenic and late vitellogenic ovaries (P>0.05, Figure 9A).

In contrast, the expression level of *PmCap1-l* was comparable in different stages of non-ablated shrimp (P>0.05). However, it was up-regulated in mature ovaries (stage IV) in eyestalk-ablated broodstock (P<0.05). The expression level of *PmCap1-l* in each ovarian stage of eyestalk-ablated shrimp was significantly greater than that in the same stages of non-ablated shrimp (P<0.05, Figure 9B).

***In vitro* Expression Levels of *PmPkaC1* and *PmCap1-l* mRNAs in Ovarian Explant Culture Treated with 17α-20β-DHP**

The relative expression level of *PmPkaC1* (Figure 10A) and *PmVtg1* (Figure 10C) in cultured ovarian explant treated with different concentrations of 17α-20β-DHP (0.1, 1.0 and 10.0 µg/ml 17α-20β-DHP) was not significantly different from the control at all time-intervals (P>0.05).

For *PmCap1-l*, its expression levels among the control and different treatment were significantly different in ovaries treated with 1.0 and 10 µg/ml 17α-20β-DHP at 1 hpt (P<0.05). Similar results were observed at 3 hpt in ovaries treated with 0.1 and 1.0 µg/ml 17α-20β-DHP (P<0.05). while the effect was not significant between the control and 10 µg/ml 17α-20β-DHP (P>0.05). Non-significant results were observed in subsequent time intervals (at 6 and 12 hpt). In contrast, the level of *PmCap1-l* in ovaries treated with 0.1 and 10 µg/ml 17α-20β-DHP was significantly greater than that of the control at 24 hpt (P<0.05) (Figure 10B).

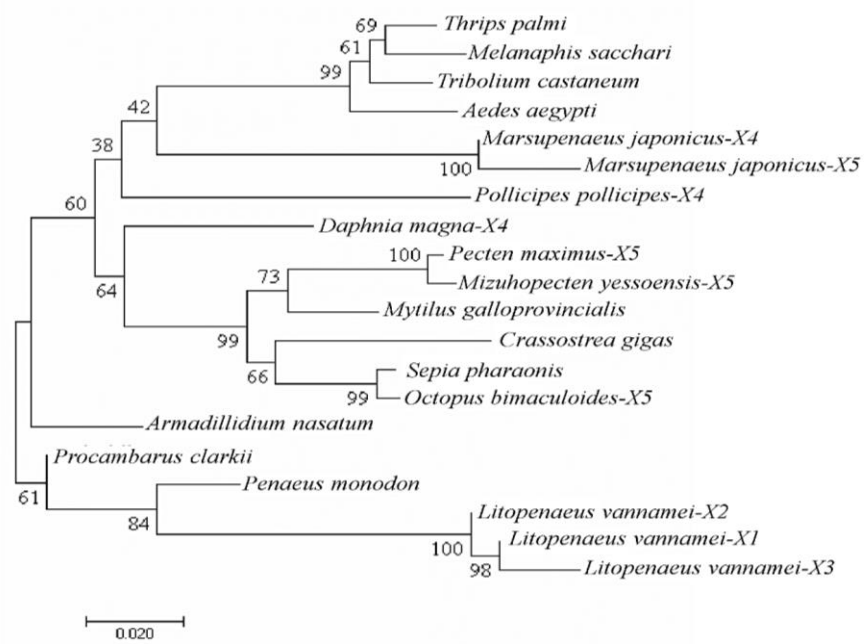

Figure 3. A bootstrapped neighbor-joining tree showing phylogenetic relationships betweem *PmPkaC1* and *PkaC1* genes from various taxa.

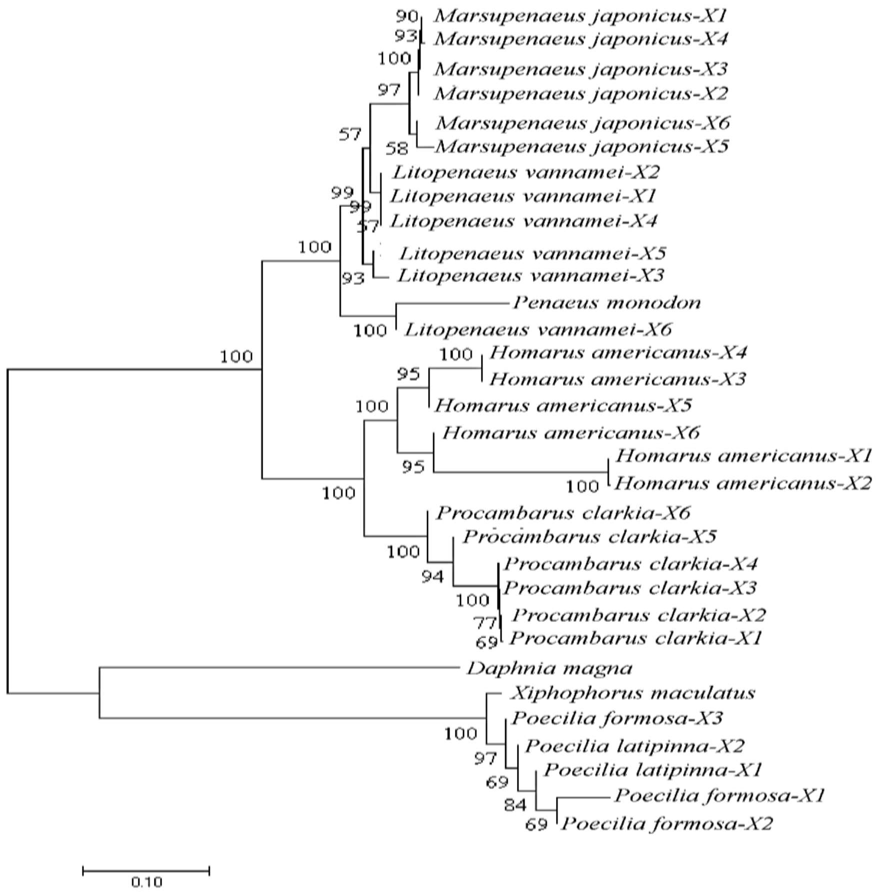

Figure 4. A bootstrapped neighbor-joining tree showing phylogenetic relationships of *PmCap1-l* and *Cap1-l* genes from various taxa.

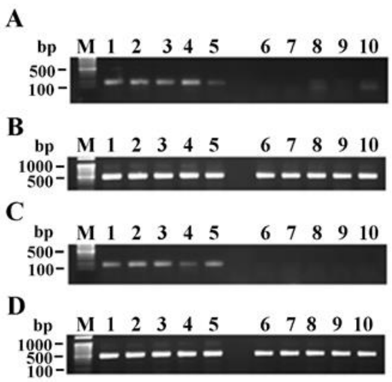

Figure 5. RT-PCR of *PmPkaC1* (A and C) and *EF-1α* (B and D) using the first-strand cDNA of ovaries (lanes 1-5, A-D) and testes (lanes 6-10, A-D) of cultured juveniles (A and B) and wild broodstock (C and D) of *P. monodon*.

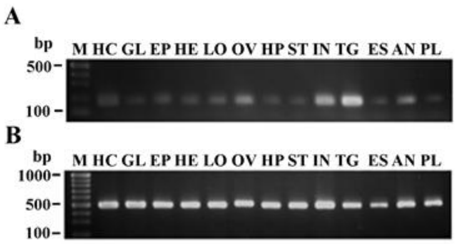

Figure 6. Tissues distribution analysis of *PmPkaC1* in wild female *P. monodon* (A). *EF-1α* was successfully amplified from the same template (B). HC = hemocytes, GL = gills, EP = subcuticular epithelium, HE = heart, LO = lymphoid organ, OV = ovaries, HP = hepatopancreas, ST = stomach, IN = intestine, TG = thoracic ganglion, ES = eyestalk, AN = antenna gland, PL = pleopod. Lanes M = 100 bp DNA ladder.

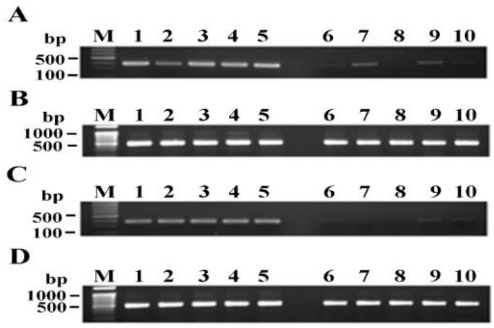

Figure 7. RT-PCR of *PmCap1-l* (A and C) and *EF-1α* (B and D) using the first-strand cDNA of ovaries (lanes 1-5, A-D) and testes (lanes 6-10, A-D) of cultured juveniles (A and B) and wild broodstock (C and D) of *P. monodon*.

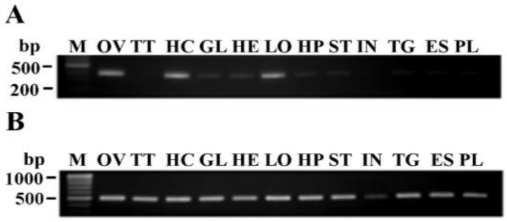

Figure 8. Tissues distribution analysis of *PmCap1-l* in wild female and testes of wild *P. monodon* (A). *EF-1α* was successfully amplified from the same template (B). OV = ovaries, TT = testes of wild male, HC = hemocytes, GL = gills, HE = heart, LO = lymphoid organ, HP = hepatopancreas, ST = stomach, IN = intestine, TG = thoracic ganglion, ES = eyestalk, PL = pleopod. Lanes M = 100 bp DNA ladder.

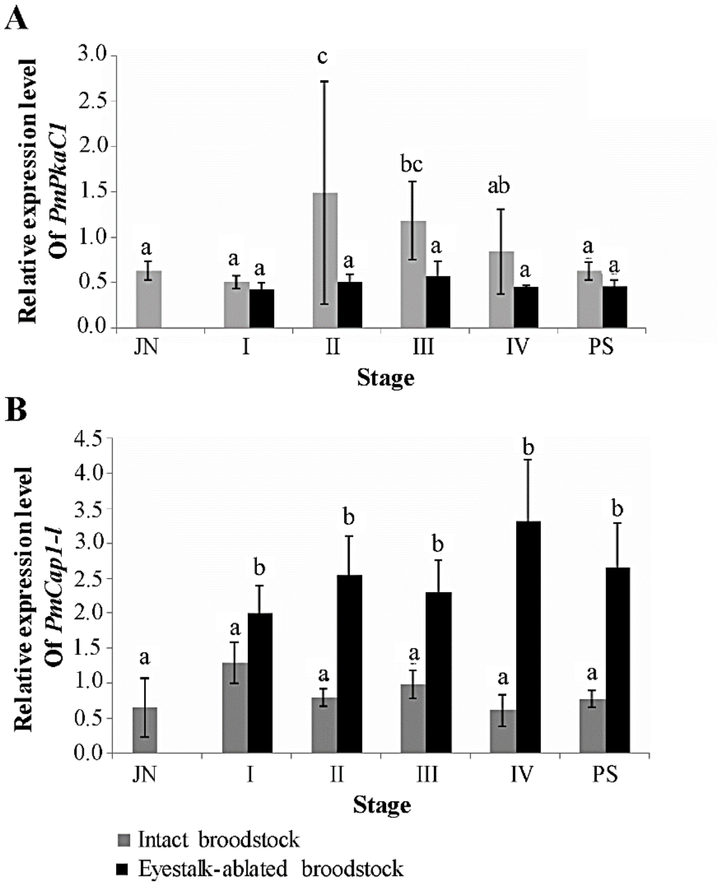

Figure 9. Histograms showing mean relative expression levels of *PmPkaC1* (A) and *PmCap1-l* (B) during ovarian development of non-ablated (intact) and unilateral eyestalk-ablated broodstock of *P. monodon*. Bars labeled with different letters are significantly different (P<0.05) while those with any letter in common are not. JN = juvenile ovaries; I–IV = previtellogenic, vitellogenic, late vitellogenic, and mature ovaries, respectively; PS = ovaries of non-ablated adults immediately collected after spawning (stage V).

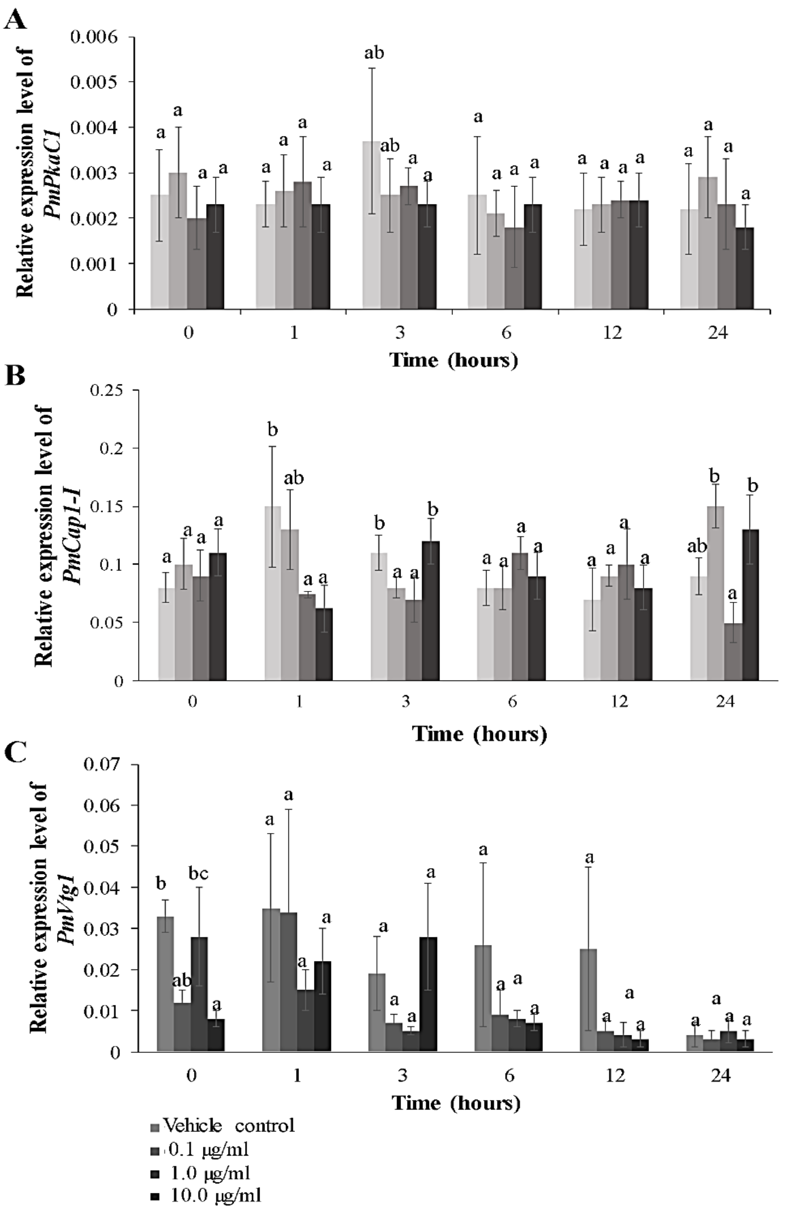

Figure 10. Relative expression levels of *PmPkaC1* (A), *PmCap1-l* (B) and *PmVtg1* (C) mRNAs at different time intervals after ovarian tissues were treated with different concentrations of 17α-20β-DHP (0.1, 1.0 and 10 μg/ml) in comparison with the untreated vehicle control. The same letters above bars indicated that the gene expression level was not significantly different (P>0.05).

Discussion

Genetic improvement is crucial for sustainable culture of *P. monodon*. This activity needs closed cycle culture of domesticated shrimp (Makinouchi & Hirata, 1995; Clifford et al., 2006; Coman et al., 2006). Development of methods to resolve the major constraint on reduced reproductive maturation of domesticated *P. monodon* requires the understanding on molecular mechanisms and functional involvement of DEGs during the ovarian development process (Benzie, 1998; Withyachumnarnkul et al., 1998; Uengwetwanit et al., 2018; Klinbunga et al., 2020).

During meiotic maturation of oocytes, meiotic resumption of fully-grown oocytes occurred and cytoplasmic and nuclear compartments need further development membrane for successful fertilization (Reader et al., 2017). The signal transduction pathways play a key role during oocyte maturation (Kishimoto, 1999 and 2003; Voronina & Wessel, 2003; Takeda et al., 2018). Protein kinases which contain the S_TKc functional domains act on phosphorylation of a protein substrate affecting the target protein functions (Pamela et al., 1991; Hanks & Hunter, 1995). Cyclase-associated proteins (CAPs) are highly conserved actin-binding multifunctional proteins that contain several structural domains (Freeman & Field, 2000; Hofmann et al., 2002; Hubberstey & Mottillo, 2002; Deeks et al., 2007).

The cAMP-protein kinase A (PKA) signaling pathway is important for the regulation of cAMP levels which are necessary for the progression of meiotic maturation of oocytes (Matten et al., 1994). In addition, changes of intracellular cAMP levels are regulated by the adenylyl cyclase or phosphodiesterase families of enzymes, (Soderling & Beavo, 2000; Sunahara et al., 1996). In this study, *PmPkaC1* and *PmCap1-l* cDNAs were isolated. The deduced PKACB protein contained S_TKc and S_TKX domains while the deduced PmCAP1-l contained CAP_N and CARP domains as commonly found in previously isolated orthologous proteins of various species (Pamela et al., 1991; Hanks & Hunter, 1995; Freeman & Field, 2000; Hofmann et al., 2002; Hubberstey & Mottillo, 2002; Deeks et al., 2007).

In the present study one type of nucleotide sequence of *PmPkaC1* and *PmCap1-l* was identified in *P. monodon*. However, multiple isoforms of *PkaC1* and *Cap1-l* genes were reported in various species. Phylogenetic analysis clearly suggested close relationships between deduced amino acids of these genes in *P. monodon* and other crustaceans but distant relationships with those from different phyla. Interestingly, *Marsupenaeus japonicus PkaC1* isoforms X4 and X5 were allocated into the same clade as *PkaC1* of various insects and mullusc species but in a different clade with that of *L. vannamei*, *P. monodon* and *Procambarus clarkii*. For *Cap1-l*, different subgroups of multiple isoforms were found in *Marsupenaeus japonicus*, *L. vannamei*, *Homarus americanus* and *Procambarus clarkii*. *PmCap1-l* was clustered with *L. vannamei Cap1-l* isoform X6 and should be recognized as this isoform. Accordingly, additional isoforms of *PkaC1* and *PmCap1-l* should be further isolated and characterized.

The basic information on a consequent effect of eyestalk ablation and hormonal treatment are important for designation of further studies of gene/protein function. *PmPkaC1* and *PmCap1-l* were more abundantly expressed in ovaries than testes in both juveniles and adults suggesting that these transcripts play more important role in ovarian than testicular development. In female adults, high expression of both *PmPkaC1* and *PmCap1-l* than other non-reproductive tissues further suggested the functional contribution of these genes in reproduction of *P. monodon*.

Typically, oocyte maturation is mediated by a reduction in cAMP levels (Conti et al., 2002; Kishimoto, 2003). In contrast, maturation of pig, sheep and rabbit oocytes require a transient increase rather than a decrease in cAMP levels. Similarly, treatments that increase cAMP levels can induce oocyte maturation in jellyfish (Takeda et al., 2006). Currently, there has been no reported on correlation of the cAMP levels and the progression of oocytes in *P. monodon*. PKA is regarded a potent inhibitor of meiotic maturation of oocytes (Schmitt & Nebreda 2002). It has been reported that a decrease in the level of cAMP attenuate the activity of cAMP-dependent PKA leading to diminish phosphorylation of proteins inhibitory to meiotic maturation (Khan & Maitra, 2013). Maller & Krebs (1977) demonstrated that injection of the PKA regulatory subunit (PKA_r), or a PKA inhibitory peptide, was sufficient to induce maturation. In addition, injection of the PKA catalytic subunit (PKA_c) into oocytes prevented maturation by progesterone. The expression profiles of *PmPkaC1* in eyestalk-ablated compared to non-ablated broodstock further confirmed that *PmPkaC1* negatively affects the development of ovarian development of *P. monodon* as previously reported in *Xenopus* (Matfen et al., 1994). In the next study, experiments on RNA interference (RNAi) should be further performed to evaluate whether the reduction of *PmPkaC1* in vitellogenic stage affects ovarian development and maturation of *P. monodon* or not.

Comparing with non-ablated broodstock, the expression level of *PmCap1-l* in each ovarian stage of eyestalk-ablated shrimp was significantly greater than that in the same stages of non-ablated broodstock ($P<0.05$). This information suggested that the reduction of GIH levels (from eyestalk ablation) directly affected the increased expression levels of *PmCap1-l* mRNA.

In our previous studies, *in vivo* effects of progesterone (0.1 µg/g body weight) injection on expression of various genes in ovaries of domesticated (14-month-old) *P. monodon* were examined. Among reproduction-related genes examined (*PmFAMeT*, *PmCOMT*, *PmBr-c Z6*, *PmBr-c*, *PmPgmrc1*, *PmCytB5*, *PmSARIP1*, *PmG$_{\alpha s}$*, *Pm17β-HSD*, *PmPkaC1* and *PmADRP*), only the expression of 3 transcripts were significantly altered including *PmPgmrc1* (up-regulation at 72 hpi; Prechaphol et al., 2010), *PmG$_{\alpha s}$* (down-regulation at 24 hpi; S. Klinbunga, unpublished data) and *PmADRP* (up-regulation at 48 hpi, Sittikankaew et al., 2010). 17α-20β-DHP is regarded as the MIH of several fish species (Takeda et al., 2018). To assess more detailed information on the molecular mechanisms of hormonal induction by a progesterone derivative, *in vitro* effects of 17α-20β-DHP were examined.

Ovarian maturation of *P. monodon* results from rapid synthesis and accumulation of vitellogenin (Yamano et al., 2004; Hiransuchalert et al., 2013). Accordingly, the expression level of *PmVtg1* was included in the experiment. However, *PmVtg1* in cultured ovaries treated with different concentrations of 17α-20β-DHP (0.1, 1.0 and 10.0 µg/ml) was not significantly different from the control during the incubation period of 0-24 h. Similar results were observed for *PmPkaC1* where its expression level in cultured ovaries treated with different concentrations of 17α-20β-DHP was not significantly different from that of the control at all time-intervals. However, the expression level of *PmCap1-l* in ovaries during the short term exposure (1 and 3 hpt) of 17α-20β-DHP was lower than that of the control. Longer exposure period (i.e. 24 hpt) resulted in the up-regulation of *PmCap1-l* in cultured ovarian explants treated with 0.1 and 10 µg/ml 17α-20β-DHP. Consequently, effects of 17α-20β-DHP on the alteration of cAMP levels in ovaries of *P. monodon* should be further investigated. Large standard deviations were observed between sample groups.

Accordingly, results from the preliminary evaluation on effects of a progesterone derivative, 17α-20β-DHP should be taken with caution.

Ovaries are functionally important in reproduction and secretion of hormones for growth and developmental regulation (Voronina & Wessel, 2003; Preechaphol et al., 2007). Appropriate oocyte development allows oocytes competence for maturation and fertilization. This requires the accumulation of organelles, metabolites and maternal RNAs during the development process (Reader et al., 2017). In the present study, *PmPkaC1* and *PmCap1-l* cDNAs were characterized. *PmPkaC1* and *PmCap1-l* seem to play a role on oocyte/ovary development and maturation in *P. monodon*. Their expression profiles during ovarian development of non-ablated and ablated wild broodstock revealed the possible functions as negative or positive regulators for ovarian development in *P. monodon*. The basic information allows the application to apply the dsRNA approach for functional studies of *PmPkaC1* to determine whether the inhibition of its expression results in the stimulation of ovarian development of *P. monodon*.

Ethical Statement

All authors declare that the present study was conducted in an ethical, professional and responsible manner following the regulation for animal care and use for scientific research of the National Center for Genetic Engineering and Biotechnology (BIOTEC) Animal Welfare Committee.

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Author Contribution

S.K. conceptualization, provided critical comments and final approval of the manuscript, K.S., S.P., P.R. and O.R. performed the experiments. S.J. and P.P. analyzed the data. B.K. supervised the findings and reviewed the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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