



Molecular characterization and expression profiles of cyclin A and cyclin B during ovarian development of the giant tiger shrimp *Penaeus monodon*

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ABSTRACT

The meiotic maturation of oocytes is regulated by maturation promoting factor (MPF), a complex of cdc2 (Cdk1) and cyclin B and other Cdk/cyclin complexes. To better understand molecular aspects governing reproductive maturation of the giant tiger shrimp (*Penaeus monodon*), the full length cDNAs and genomic organization of cyclins A and B (*PMCyA* and *PMCyB*) were characterized. A single form of *PMCyA* contained an open reading frame (ORF) of 1326 bp corresponding to a deduced protein of 441 amino acids. Its genomic sequence contained 5 exons, 4 introns and untranslated regions (UTRs) spanning 2586 bp in length. In contrast, *PMCyB* possessed three isoforms with an identical ORF of 1206 bp (401 amino acids) but three different 3' UTR lengths of 416, 543 and 1117 bp, respectively. Their respective genomic sequences were composed of 8 exons, 7 introns and UTRs covering 4181, 4307 and 4940 bp. Expression levels of both *PMCyA* and *PMCyB* in ovaries of broodstock were much greater than those of juveniles ($P < 0.05$). During ovarian development and after spawning of normal shrimp broodstock, *PMCyA* was not differentially expressed ($P > 0.05$) whereas the level of *PMCyB* in stage IV was greater than that of stage I ovaries ($P < 0.05$). Unilateral eyestalk ablation, a technique commonly used to induce spawning in *P. monodon* female brooders, had no effects on transcription of *PMCyB* ($P > 0.05$) but resulted in a lower expression of *PMCyA* at stage IV of ovarian development of this economically important species ($P < 0.05$).

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1. Introduction

During oogenesis, immature oocytes resume meiosis and fertilization occurs at a particular stage of oocyte maturation depending on species (Kobayashi et al., 1991; Okano-Uchida et al., 1998). Progression through the cell cycle is regulated by association between cyclin-dependent kinases (Cdks) and their cyclin partners (e.g. cyclin A and cyclin B) governing at different points in the cell divisions (Westendorf et al., 1989; Knoblich and Lehner, 1993).

Cyclins play important roles in the reproductive development of eukaryotes. In all animals, oocyte maturation is regulated by the complex of cdc2 (Cdk1) and cyclin B (called maturation promoting factor, MPF). In various species, MPF has been elucidated to be involved on molecular resumption of arrested oocytes (Kobayashi et al., 1991; Kishimoto, 1999, 2003).

Both cyclins A and B bind to Cdk1 and synergistically allow cells to proceed through the G2-M checkpoint (Minshull et al., 1990; Lees and Harlow, 1993; Li et al., 2004). Nevertheless, they essentially confer different intrinsic roles during the M phase (Knoblich and Lehner, 1993). For instance, cyclin A2 regulates breakdown of the nuclear envelope and the

accumulation of cyclin B1 (Jackman et al., 2003) but cyclin B1 implicates centrosome separation during the cell separation event in both mitosis and meiosis (Gong et al., 2007; Voronina et al., 2003). In addition, cyclin A also forms a complex with Cdk2 to promote the G1-S phase transition for the chromosome replication at the end of meiosis (Pagano et al., 1992) and to potentiate the transcriptional activity of genes encoding estrogen and progesterone receptors (Rogatsky et al., 1999; Narayanan et al., 2005).

Reduced reproductive maturation of female giant tiger shrimp (*Penaeus monodon*) in captivity prevents effective domestication and selective breeding programs for genetic improvement in this species (Preechaphol et al., 2007). In the present study, the full length cDNAs and genomic organization of *P. monodon* cyclin A and cyclin B (*PMCyA* and *PMCyB*) were isolated and characterized. The former was reported for the first time in crustaceans. Expression patterns of *PMCyA* and *PMCyB* during ovarian development of normal and eyestalk-ablated *P. monodon* were examined by quantitative real-time PCR.

2. Materials and methods

2.1. Experimental animals

Juvenile shrimp (*P. monodon*; 4 month-old) were purchased from a commercial farm in Chachoengsao (eastern Thailand, $N = 5$). Female

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A

ACGGGCGCGGGAACTATCCTCACTGGCGCGTCAATAGTGTGCGGGTCTGTCTTCCTGTTGG 60
 GCCTCGGAAAGAGATCTTAAACAGAAAGGGCAACGGCGACGGAAACCCTCTCGCGAAAA 120
M
 TGGCTTCCACGCA CATATTCACGAA CCAGGAGAACGAGTCCCGCGGCCACGCGGAGAG 180
A S T H I F K D Q E N R V P A A Q R R G
 GCAAGAGAGAGGACCCGAGGGCGAGCAGTGTCTTTTAAACAGACGCCAAGTGTCTCGGGT 240
K R E D P R A S S V L V N R [R P V L W G A]
 CTATCAACCCCAATTTGCGSAAACAACAGCTAGACAGCCAAACAGGGGTGTAGTATG 300
[E] P N L R K Q P A R A A K Q G V S Y E
 AAACTGGGTATTTCCACAGCAAGCTGAGAAATGCCCTGCCCTCACAGAAATCTTTCTCGA 360
T G I P Q A G A D E N A C P T Q K S F S T
 CAAGTCAA CCTCTTTACTATTCATGAAGATGCACTGCTTCACTGGGAACACCAAGTA 420
S Q P S F T I H E D A C L S M G T T S N
 ACAGCCAGAGTGCATCTAGCAATTCACAGGACTAAACCCATTAAGTGAAGGTTAAGTA 480
S Q S A S S N S T G P N P L S E R L S K
 AACCACTAACAAGTGAAGAAATCTTGAAGATTAAGCACTGTGAACAGAAATGCG 540
P L Q S E E E S I E D I D P S V T E L Q
 AGAGGTCCTATCTCGAAACAATTCAGGATCTCAAGATGAATGGAGATGAGTGTATGTG 600
R S L S I E H S G S S D V M G E S V C E
 AAGATGACCTTATGGTGTAGAAACCACTCCCGTGAAGTGTGCTTATCATAGTAGAAATG 660
D D L M V G T E T P R E D V L H S R N D
 ACGACATA TTGATGTC CCGAATA TGCAAGACACACTTAAACAGTACTGTAGAGAGCAG 720
D I F D V P E Y A A D I Y Q Y L R E A E
 AGGTGTCGCAACAGCCCGTCAAACTACATGTCAAAACGAGCAGACATCACAGCAAGTA 780
V C H K P R A N Y N M S K Q T D I T A S M
 TGAGGTGAGTCTGTGCTGACGTGGCTGTGGAAAGTGAAGGAAATACAGTCTACATA CAG 840
R W I L V D W L V E V A E E Y S L H T E
 AAACCTTTAATTTGGCTGTTTCAATATTTGACCGGTTTATAGCCAGATGTCTGTAAAAA 900
T L Y L A V S Y I A D R F L S H M S V K R
 GGTATAAATCTACAGTGGTGGACCACTGATGTTTATGCTGCTGaaactctc caact 960
D K L Q L V G T T A M F I A A
 gtttatttgggtgtgctattatggaatttctaaatgcttataatttaataccgggtgtc 1020
 tgtgctaattacaactctttccacaggaattatgaggaagacttaccctcttgcctgtaggac 1080
K Y E E I Y P P D V G Q
 AGTTTCTTATATAACCCGACA TACCTACAGATTTGGCAGATTTGAGAATGGAGCATC 1140
F A Y I T D N T Y R V G Q I L R M E H L
 TTAATTTGAAAGTGTAAAGTTTGAACATGGCAGTA GCAACTACACATTTTGTCAATA 1200
I L K V L S F D M A V P T T H L F V N K
 AGTTTCCAGAATGTGCAAAATTTGAGAAGAAACCTTTGCAATTTAGCCTTggtaggatta 1260
F A R L C K C S E E T L H L A L
 attagaatatttgagaagttgtaaaatcaataaaacagaaattttttttcaataaaaca 1320
 tgcactataacacctaactctcaattcagTCTTGGCAGAGTTACTATGCTAGAAATG 1380
F L A E V T M L E C D
 ATCCTTCTCGAGATCTCGCTTCTGTGATGCAACAATCAGCTGTTTCAATPGCAAATC 1440
P F L R F L P S V I A A S A V S L A N H
 ATACA CAGgttagttttataaattccttacagta tgaactttaaacttcagaggtcaac 1500
T Q
 taatagtgaagctt gtaataatctggaagat agttgacctgactatacaactct 1560
 ggcttgttagctcttataatcttcttcaacgggtTCACA CTGCC TGCCATCACACATG 1620
G H T A W P S H M
 GTAGAATCCACAGGATA CAGTGTAGAAACCTGAGAGATGCTATGTAAACCTACATCGT 1680
V E S T G Y S L E H L R E C Y V N L H R
 GTTFTCCACAGATTCATGAACTCAGCAACCGCTTATGAGATAAATA TAGAGGCACA 1740
V F S R V H E Q I R D K Y R G T
 AAgtaagttctcataatctaaatattccaagtttggaagattatagtggtatataatctg 1800
 aagacat acaaaagcat ggaacaattgaaatccacatttaataccaatgcttagat tat 1860
 acaaggtgacttgggtttcataatatttgacAGTGCAGGAGTGTCGCCCTTAATCCA 1920
W H G V S R L T P
 AGAAGACTCTCCCTCGTGA TGAACAAAGAGTGAATTAAGGGATGCTGATTAATGTGT 1980
R S H F P W *
 GTGAAAGTGTATAAGTAAATTTGTGTGGTGTGTTTTCGCCAAGACTGAATGGAATGTAG 2040
 CACTTTTATACAGATAAGCAAACTCGGTGATCATGAGTATATACTCGCTTTTCCACA 2100
 GTTCACTGGTTCAAAGCTTGGTGCACGTTTCCAGTGAAGCGTGGAAACAGTGTGATGAG 2160
 GCCTCTGGTAGAGTAAGGAAATTTATCTTCAACAGGATGCTTATCAAGTGTACAGGTT 2220
 AATTTTATTAATAA CCTTTTATATTAATTAATGAGTCAAGAAATCA CAGCTTTAA 2280
 AAGGCCCTGTAGCAGATCTCTTACGGTGTGAACGTCACTGCTTACCAGTCACTGCA 2340
 TGATA TTAGAGGTTGCTCATAGCAATGATTTTCAATTTTGTGATGTAAGGAATGCC 2400
 TCGTGTGATACCTTGTGCTGCAAGAAACATATCAA GTTTCAGMAAGACATGACAAATG 2460
 GCTGTATA TATGTAAC TAGAACTCTGAAAGATGAAGGATTTTATCTCTGATTTTAA 2520
 CCGAAGACACTTCCAAAATACATTTCTTCAGATTCAAA AAAAA AAAAA AAAAA 2580
 AAAAAA 2586

B

GGGAGTGTCSAGT CGCGTCTGCTTGAGAGAGGACCTGTTTGAAGGATTTCCGACGTGA 60
 AAAAAGCCACACA TTCTCGAATATCCGGTTGATTTTATTCATACTAGTCTTTGAGAA 120
M S L R L T T
 CCA CGCATCTCAA CAGTgta agttcca aga cggggaa gacggcgagaa agg 180
T H L N S
 cggaaattgt aacgt tgcggcgccacgaggtctgggtctcgcgcccggcgtt taaatgcc 240
 ccggaatttaactcttgtctccacttggcggcctcgggtccacttcaatttttttttttca 300
 tttctatttccctagcaactata tgcggccatcgggtat ttagaacctccgaagtgcggctt 360
 cccgtacggggatcgggaatatttattattaggaatattggaattgggaatctctgtggc 420
 ggttattatttgttttctgtatgttttttaccctctctgctgttactctgca 480
 tctgtttttatggtttgatgtctgtaattgacggcctcaactctttcactgagaagaat 540
 caacttgctttgtaatttgaatatttaaaatgacttgggaatgactgttgaatggcct 600
 acttggtttgtaattctctgtgtaatacgggaatgactgttggggctctattaaaggtgtgat 660
 tgcactccctaa gtttttaattaa ggtgagggaaactggtttttctctctttctgtcgt 720
 gggaaatagttttctaggttttaaattatcaaaactttttttccctctagtagaacctggg 780
 aat tgaagctcaacttttttaaatgtttaaatacaactggtttttctccaaatgactgtgca 840
 gcttactctactgtgtagaaagcggcgtcaagcagctctctggacgttctttttggac 900
 agtgaattcagtcgaaagattatgacttctctgttactctgttactaat tgcctagggaggt 960
 atcagctcgtgtaagttgttctccctcaccaaggtgtagggctacgtgtagagaggtt 1020
 agagaatgggtgttttggatttttggatcaacagacac tttgagattttggttcaaca 1080
 agcctaaaagtaaacctctttctcttttaaatattccactgactctctctttctccag 1140
 AACCTGGGCGACACTCAACAATCCCGCAAGTGAAGGCCAAAATGATCCAGGGGCCA 1200
N L G H G T D L N N P R K V E A K M I Q G P
 GTCACCTGTGTGCGTGTGTGGATTTGGCAACCGTCCACTCTGCAAGGCCCCAAA 1260
V T [R A R F V D V G N] R A I P V G Q P K
 CCTCCCTCAAGCTGGGGGAGTCTCCGAAATGAATCGTGTGAAGCTGCAAGGCCAAA 1320
 CCCCCCTCTGTGGGCTGCTCGCAAGTggggaactttttctctctgggggtaaggga 1380
A G L S G L L A R
 ggttcatatataaaggagttcttggagactggttaattctattctggcgggcaataa 1440
 gtgaagggctcgggggagcagaactctcagcaaatctcaagcaagcagctggttaattctta 1500
 gtaagggctatcggaggaggtgtagtccgaaatggtattctcgtgactgagctggtctct 1560
 tttttgattgtagtctcgaagaaatggttcgataattctgctatcggattatataatttt 1620
 ttgtattttgtagtcaactcggcaagaaatgtagagccctcaaggaagtggttagagcattg 1680
S G K E N V K P L K E V E H V
 TGGAGCAGATGGA TGGGAGGAGGAAACCAAGGTGGAGAGCTGCTATTGCTTTCTCTA 1740
E Q M D V E E A G V E E L A I A F S T
 CCCAGAGACTAGATGTTGAAGATATGATGCCCAAGACAGTGAATAATCCTCAGCTGTAT 1800
Q R L D V E D I D A Q T S D N P Q L V S
 CTGAAATGATGAATATCTCAAGTACCTCGAGGCTGGAGgtaacagacctggtgat 1860
E Y V N D I Y K Y L R E L E
 ctgggttgggaatactgtgtagaaactctgttctgtctgtttaaagaagtttagtgattatgta 1920
 ttttatgccaagcagactaatcatgtggaacaactgaactcgggtgtgcaaacagctgtg 1980
 cgtggaaataataaatactcagactcaggcgggtgtgcaactaaagagtgtaataattggt 2040
 agtataagcttagaaacaaaacaaatccataatgtagtttagatgcttaaatattgtgctgt 2100
 gttggtgcaactctgcaacgagctggtggtgaaacccagcggctcttctgcaactcgtggg 2160
 gtagtttggagatcaataaacacagcagctttttctctctctaacagGATGCCAACAA 2220
D A N K
 AGTCAAGCCAGATACTTAGAAGGCGCAAGTAA TTA CAGGAAAGATGAGGACATTTGAT 2280
K P R Y L E G Q V T I T G K M R A I L I
 TGA CTGCTGCTGCAAGTACACTCGCTCTCACTCTGCTTCAAGAAACACTGTATCTGAC 2340
D W L V Q V H L R F T L L Q E T L Y L L T
 TGTGTGCTATCATTGACAGATTTCTCCAGGtaactagatggttattactctcttatttaa 2400
V A I I D R F L Q
 tttttattttttctgtttttgtaataactttttctcttttacaatttttaaaagg 2460
 tttcttaccactccagACTCAGAGGAATA CCA CGTAA CAAGCTACAGTTAGTGGTG 2520
T Q R N I P R N K L Q L V G V
 TGA CTGCCATGTTCTTCTAGCAAATATGAA GAAATGATGTC CCA GAAATCGGGACT 2580
A M F I A S K Y E E M Y C P E I G D F
 TCGCATACATCAGACAAA GCCTACTCAAAGCCAGATTCGPTAAATGGAAGGTGCCA 2640
A Y I T D K A Y S K A E I R K M E V T M
 TGCTGAAATGACTGGGCTTCAAATGATCTTCTTCCCTTACACTCTCTCGAAGAA 2700
L N E L G F N V S Y P L P L H F L R R N
 ACA GAAACCTGGCTCTgtagtactgagtagtatttggtatgtagatgcaatattactcc 2760
S K A G S
 tataaataaaactactgatactgctctgattgtagtagaaactagtaattctgtgtcaac 2820
 agtggtagcttttcaaacacacacttggcgaagtagctttagtgaactttgcttccagagat 2880
V D A S Q H T L A K Y L M E L C L P E Y
 ACAGCATGTGCCAATACAAGTGTCAA TGA TTTGCTGCATCTGCTCTGCTTCACTTA 2940
S M C H Y K S S M I A A S A L C L S L K
 Agttagatataagattttaaactaggactctcaagtgacattaaattttactcgtct 3000
 cctagattgcaagttaattctgctttaaattgctacttattgttttaatacagctctc 3060
 atgtgtttattgtagtctttcttaagtgaatggatgtgcaataattgctccatc 3120
 attgtagtctagtcagagatcacctcttttgcctattgaagaagaa aaaaaaaa 3180

aaaactaaaaaaaactatctggtgaggaatttggactatttctttaaattatctatccc 3240
 attaaaaattctctccccacacttcaagtaatttttattttctttaggtttgtttggat 3300
L L D
 GGCATAA CTGGAGTGA TACATGACTTCTTACTTCTCGCTACACTGACAACAGCTCATD 3360
G N N W S D T L T F Y S R Y T E Q Q L M
 CCACTCATGTGCAAAATGGCATCAGTTGTAGTAAA GAGCA GTAGTGC CAAGCAACAGTga 3420
P V M C K M A S V V V K S S A K Q Q
 agagtaatttttagctttgttcttatataaaagaaatatttttgataagtagggcac 3480
 ttttaaaatagaatttagactggttttactgttcaaaatgtgtacattttgaaactca 3540
 ctatgcttataacagtttggcttacttgcgaatggagatgcaagcttgcaactgtctca 3600
 ttttcaatccccctgtataggtctgacccctagcttataagctgttttggtaaaagag 3660
 tctgttaatttagtatttacttgcagGCTTAGTACAGATGATCAAAAGCTGCAAGTGTG 3720
A V R Q K Y K A S K L
 ATGAAAAATTAGTGAGATTTCCCAGLCTCAAATGCAAGCTCAACTCAACTCGCAGAGAA 3780
M K I S E I P Q K T S K L I N S L A E K
 AGTGCCTTTATGATGATGAGGTGGTGGCCATTATAAAGTAAATATTGTACATGTTGAATG 3840
S A S Y A *
 AATGGACTGGTTTTTGTACATA TTGCTTTAAGATGCCAGTTTTCCTTTTTCATAAGCTTT 3900
 CAGATTTATAAATACTGACTACACTGACTAGTATTAATTTTATTTTAA CAAGA CTAGTCTCT 3960
 GGGTACGATGTA CAACCCAGAGTGCCTGGTTTGGCATCTATCCATTTATCATGCTCT 4020
 CATTTGATTAATA TAA TTTAGACAGGACAAAATGGCTATTGTAGAAAGGAACTTGA 4080
 AAGAAGATATAGCTATAATCTGTGTAAAGTATATATGATCTGTGATAAATCTTTAGTA 4140
 GTTCTGTATGATACTTATATAATAAACACAGTTTATCCAAAGTGTGATTCCTCACTATT 4200
TTTTTGGCTCTTAGTATACATTTGCAACA TAAGTGGATTTGACAGTAAATAGTGTAT 4260
 TTTAGATATTGCTTTATAA CAATCTTTCTTACTAGTTTTCAGACTCACAAAGAAAT 4320
AAAATGCACTGCTTACTTTTATAAATGCBAAGCAAGCCCTTGTGGCCAAATATTGTA 4380
ACA TTTTATTTCTTTTATTTAGAACTCGATGTTGATTTTATTTAGCTGAGGATGCAATTCCT 4440
TATGCAGATTTAGTGTCTTTCCCAAATGTGATTTTAACTGTGACTTTTAAATATTCCG 4500
TAAATTTGCA CAGGATATATTTTGTGATGTTGATGAGTTTCCGGAATA TTAGGATTAATG 4560
ACAAGGGA TCCGTTTTCCTAATA TTTTGTGTTGACTTTTGTGACAGTGTGCTTTG 4620
GATGGTAGTGTGCTCCCAATTTCTGGGCAACAGGTGACCTTGTATTTAGCCGCTGTG 4680
AATGATGATGAGTGTGGGAGAAABAATGGAACAATA TTTTCTGTTGATTTGCTCA 4740
TAGAABAATCTAGTGTCTGACCAAGGATGATTAATAATTTCTGCTGATGTTGAT 4800
GACTCAACTGACTGCTATATTTCTTCACTGATGATACTCCAGTGTGAAAGAAATTAAGT 4860
CTCTTACCCATACTACTGTAATTTTGA AAGCTAAACAATA CAGAAA AAAAAA 4920
AAAAA AAAAA AAAAA 4940

Fig. 1. Nucleotide sequences illustrating organization of *PMCyA* (A) and *PMCyB* (B) genes. Coding nucleotides and deduced amino acids of each exon are capitalized. Introns are italicized and illustrated with lower letters. Start and stop codons (asterisks) are illustrated as boldface. The N-terminal cyclin destruction box (RXALGXIXN) and the C-terminal cyclin domains are boxed and highlighted, respectively. The length of 3' UTRs of *PMCyB-m* and *PMCyB-l* extended from that of *PMCyB-s* and *PMCyB-m* is single and double underlined, respectively. Cytoplasmic polyadenylation elements (CPU, U/UUUUUUA) are illustrated in boldface and underlined. Polyadenylation signals (AATAAA) are boldfaced and italicized.

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