Comparative Biochemistry and Physiology, Part A 152 (2009) 535–543

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

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

Virak Visudtiphole [a], Sirawut Klinbunga [a,b,*], Kanyawim Kirtikara [a]

[a] Aquatic Molecular Genetics and Biotechnology Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, 113 Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand

[b] Center of Excellence for Marine Biotechnology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

ARTICLE INFO

Article history:
Received 28 October 2008
Received in revised form 16 December 2008
Accepted 16 December 2008
Available online 25 December 2008

Keywords:
Cyclin A
Cyclin B
Penaeus monodon
Ovarian development
Eyestalk ablation

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 ($P<0.05$).

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

* Corresponding author. Tel.: +66 2 6448150; fax: +66 2 6448190.
E-mail address: sirawut@biotec.or.th (S. Klinbunga).

1095-6433/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2008.12.011

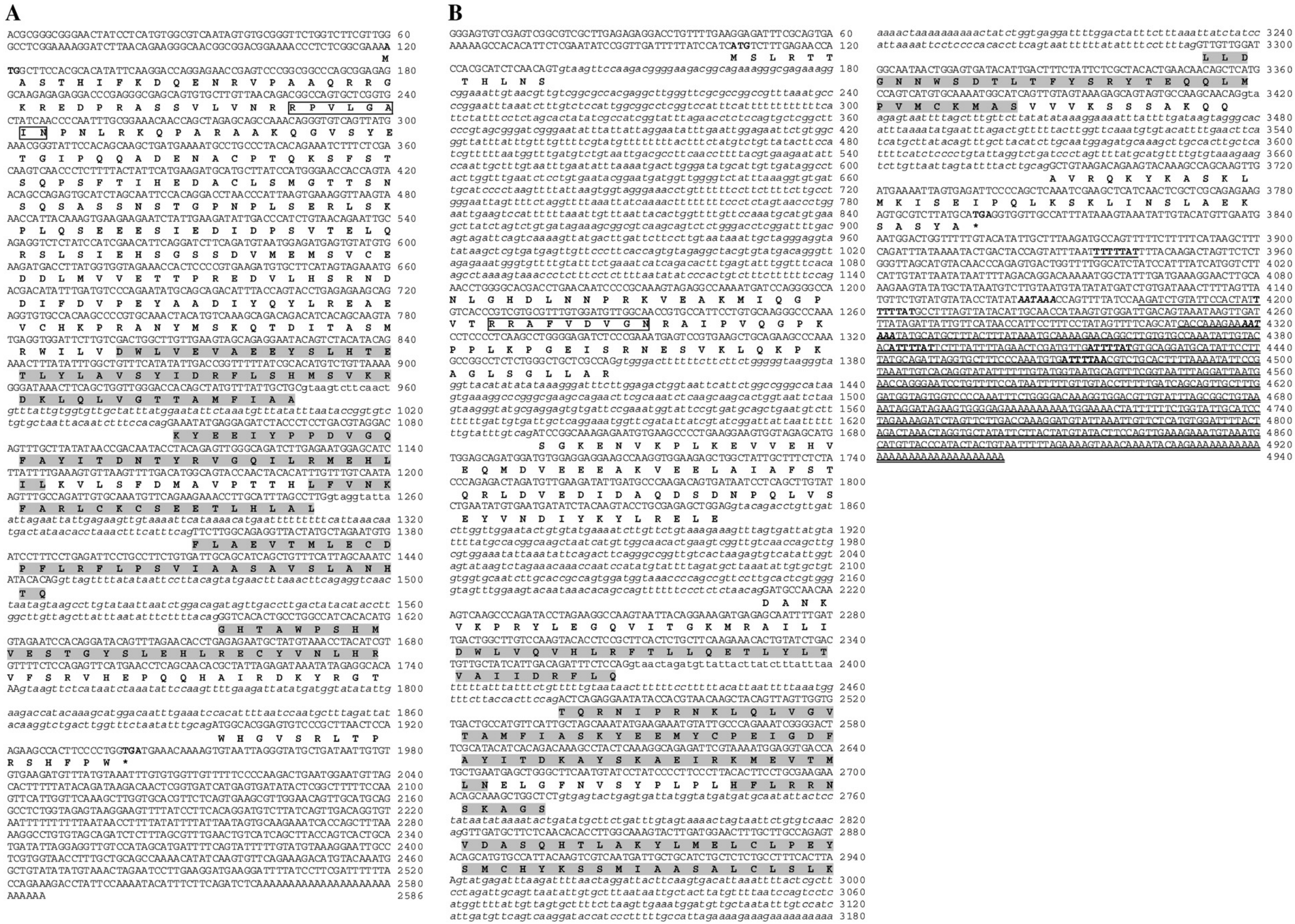

Fig. 1. Nucleotide sequences illustrating organization of *PMCyA* (A) and *PMCyB* (B) genes. Coding nucleotides and deduced amino acids of each exon are capitalized. Introns are italicized and illustrated with lower letters. Start and stop codons (asterisks) are illustrated in boldface. The *N*-terminal 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/AUUUUAU/A) are illustrated in boldface and underlined. Polyadenylation signals (AATAAA) are boldfaced and italicized.