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# Three forms of cyclin B transcripts in the ovary of the kuruma prawn *Marsupenaeus japonicus*: Their molecular characterizations and expression profiles during oogenesis

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**Abstract**

Cyclin B is a well known regulatory factor that plays a crucial role in mitosis and meiosis. Although the existence of cyclin B has been reported to be universal in a wide variety of eukaryotic organisms, no molecular data are available on crustacean species. In this study, three forms of cyclin B transcripts were first identified and characterized in the ovary of the commercially important kuruma prawn *Marsupenaeus japonicus*. The three transcripts (2.4, 1.9 and 1.7 kb) shared the identical sequence, with variations only in the length of 3′ untranslated regions (UTRs), and coexisted in the ovary as demonstrated by Northern blot analysis. The sequences of 3′ UTRs indicated that the distinct length UTRs of the transcripts is attributed to an alternative usage of various polyadenylation signals in the 3′ UTR. The open reading frame of 1203 bp encoded a putative 401 amino acid peptide. The deduced amino acid sequence shared 45–50% identities with the known B-type cyclin in other animals. Quantitative real-time RT-PCR revealed that the short transcript (1.7 kb) was the most abundant among the three transcripts, followed by the long (2.4 kb) and medium (1.9 kb), and the three forms of the transcripts displayed various expression profiles during oogenesis. In situ hybridization showed that the short transcript commenced expressing in the ova as early as the oogonia stage and accumulated largely at the perinucleolus (PN) stage, whereas almost no expression was found for the medium and long transcripts at the oogonia stage and moderate signals were detected at the PN stage. The differential expression of the three forms of transcripts suggested that various transcripts might perform different roles during oogenesis of the kuruma prawn.


*Keywords:* Cyclin B; cDNA cloning; Differential expression; Oocyte development; Penaeid shrimp; Quantification; Transcripts; 3′ untranslated regions

## 1. Introduction

Cyclin B is a ubiquitous regulatory protein that controls eukaryotic cell division cycle at the checkpoint from G2 to M-phase by combination with catalysis subunit Cdc2 kinase to form cyclin B-Cdc2 kinase complex, termed M-phase-promoting factor (MPF) (reviewed by Kishimoto, 1988; Nurse, 1990; Masui, 1992; Yamashita, 1998; Pines, 1999). MPF is also known as a maturation promoting factor, since it was initially identified and purified in mature oocytes of *Xenopus laevis* (Masui and Markert, 1971; Lohka et al., 1988). MPF was reported to be present in oocytes cytoplasm under the induction of the pituitary gonadotropin at the final stages of oocyte maturation (reviewed by Nagahama, 1987). The activation of MPF leads to M-phase entry and progression of oocytes to meiosis maturation including chromosome condensation, germinal vesicle breakdown (GVBD) and spindle formation (Masui and Markert, 1971; Mailer, 1990; Nurse, 1990). The catalysis activity has never been detected when Cdc2 kinase exists alone. Cyclin B is required for activation of Cdc2 kinase in *X. laevis* oocytes, involving phosphorylation of Cdc 2 on Thr161, and dephosphorylation on Thr14 and Tyr 15 as well (Minshull

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et al., 1990; Meijer et al., 1991; Fesquet et al., 1993; Poon et al., 1993; Solomon et al., 1993).

The cDNA cloning and characterization of cyclin B have been well documented in an extensive variety of animals such as fruit fly, teleosts, amphibians and mammals (Pines and Hunter, 1989; Westendorf et al., 1989; Whitfield et al., 1990; Chapman and Wolgemuth, 1992; Hanley-Hyde et al., 1992; Kreutzer et al., 1995; Nakahata et al., 2001; Voronina et al., 2003). Two forms of cyclin B transcripts were detected in fruit fly (Dalby and Glover, 1992) and mouse (Chapman and Wolgemuth, 1992), three in nematode (Kreutzer et al., 1995). The primary structural difference between the forms of the transcripts lies in the 3′ untranslated region (UTR), which contained translation regulatory motifs: a cytoplasmic polyadenylation element (CPE) with a consensus sequence of U/AUUUUAU/A (Sheets et al., 1994; De Moor and Richter, 1999) and a cis element (also named TCE, translation control elements) with a bipartite consensus sequence of GUUGU-$X_{23}$-AUUGUA. The CPE has shown to mediate translationally both masking (repression) and unmasking (activation) of the maternal cyclin mRNAs through polyadenylation and deadenylation during the oocyte maturation of *X. laevis* and mouse (De Moor and Richter, 1999; Paris and Richter, 1990). The bipartite sequence of TCE resembles that of a nanos response element (NRE), located in the 3′ UTR of *hunchback* and *bicoid* mRNAs, involved in translational repression of these mRNAs in the pole cells of early *Drosophila melanogaster* embryos (Dalby and Glover, 1992, 1993). Another element in 3′ UTR is required for posterior accumulation of the cyclin B transcripts in the oocyte at the late stage of oogenesis through to subsequent embryogenesis. All of these findings suggested that the 3′ UTR of cyclin B transcripts has important roles in the post-transcriptional regulation of gene expression including control of cellular and subcellular localization of the transcripts. To date, however, no data of cyclin B are available in any crustacean species and the regulation of oocyte maturation in crustaceans is still poorly understood as compared to other oviparous animals, even though the number of crustaceans ranked third in arthropod and contains many commercially important species including shrimps, prawns, lobsters, crayfishes and crabs.

In penaeid shrimp, the development of oocyte arrests at the first meiotic prophase (prophase I). After completion of yolk accumulation, the full-grown postvitellogenic oocyte resumes meiosis, the germinal vesicle begins to disintegrate and migrates towards the peripheral cytoplasmic membrane from the center of oocyte. Eventually, the mature oocyte arrests at the first meiotic metaphase (metaphase I) until fertilization or activated artificially by chemical agents (Anderson et al., 1984; Clark et al., 1980; Yano, 1998). It remains unknown whether crustaceans possess a gonad-stimulating hormone or gonadotropin homologue that can trigger the meiotic resumption during final oocyte maturation as those in most vertebrate animals such as mammals, fishes and amphibians. Additionally, there is no report associated with the characterization of MPF and its regulatory mechanism of final oocyte maturation, which is obviously an important aspect towards artificial control of maturation of the commercially important species and will contribute to a sustainable production and increase in natural resources. In the present paper, three forms of cyclin B transcripts were identified and their differential expressions during oogenesis were examined in the kuruma prawn, *Marsupenaeus japonicus*. The cloning and characterization of cyclin B cDNAs will provide an efficient approach to investigate molecular mechanism of oocyte maturation in penaeid shrimps.

## 2. Materials and methods

### 2.1. Animals and ovarian tissues

Kuruma prawns (*M. japonicus*) were purchased from a local fisheries agency (Anori in Mie Prefecture, Japan) and transported directly to our laboratory for surgically dissecting. A small portion of ovarian tissue was put into liquid nitrogen and stored at −80 °C. Another piece of ovary was fixed in Davidson's fixative (30% ethanol, 22% formalin, 11.5% acetic acid) for histological observation. The growing oocytes were staged according to histological characteristics as described in our previous study (Qiu et al., 2005). The most advanced stage of oocytes in the ovary represents the stage of ovary, since the ovary contains various stages of oocytes. For hybridization in situ, ovarian samples were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) solution overnight at 4 °C and stored in methanol at −20 °C after washing with PBS four times at room temperature.

### 2.2. RNA isolation

Total RNA was prepared from approximately 100 mg of ovarian tissues at various developmental stage of oocyte by using either RNA Extraction Kit (Amersham Pharmacia Biotech) or Trizol regent (Invitrogen). Poly (A)+RNA was isolated from total RNA using mRNA Purification Kit (Amersham Pharmacia Biotech).

### 2.3. Degenerated RT-PCR

First strand cDNA was synthesized using an Oligo$(dT)_{12-28}$ primer (Amersham Pharmacia Biotech) and PowerScript Reverse Tanscription Kit (Clontech) following the company's instruction. PCR was performed using a pair of degenerated primers, 5′-ATT/A GCA/T AGT/C AAA TAT/C GAA GAA/G ATG TA-3′ as a forward primer, and 5′-TC CAT IAG/A G/ATA T/CTT T/GGC A/TAG/A IGT ATG-3′ as a reverse primer. PCR reaction cocktail with a total volume of 25 μL contained 1 μL first strand cDNA, 2.5