Molecular cloning and expression analysis of the *ATP/GTP binding protein* gene in the giant tiger shrimp *Penaeus monodon*

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Abstract

Isolation and characterization of genes functionally involved in ovarian development is necessary for understanding molecular mechanisms of ovarian development in the giant tiger shrimp (*Penaeus monodon*). In this study, the full-length cDNA of *P. monodon ATP/GTP binding protein* was characterized by primer walking. It was 1547 bp in length containing an ORF of 1263 bp that corresponded to a polypeptide of 420 amino acids. This sequence significantly matched *Pre-mRNA cleavage complex II protein (Clp1)* of *Harpegnathos saltator* (*E*-value = 1e-155). The calculated isoelectric point (p*I*) and molecular weight (MW) of the deduced amino acid sequence of ATP/GTP binding protein of *P. monodon* was 5.98 and 46.52 kDa, respectively. The pre-mRNA cleavage complex II protein (Clp1) domain was found at positions 149-481 of the deduced ATP/GTP binding protein (*E*-value = 5.50e-157). Quantitative real-time PCR revealed that the expression level of *ATP/GTP binding protein* in intact broodstock of *P. monodon* was up-regulated in ovaries of post-spawning shrimp ($P < 0.05$). However, the expression of *ATP/GTP binding protein* was not significantly different in eyestalk-ablated broodstock. In addition, the expression levels of this gene in the same ovarian stages of both intact and eyestalk-ablated broodstock were not significantly different ($P > 0.05$). The information suggested that gonad inhibiting hormone (GIH) does not exhibit the downstream effects on the transcription of *P. monodon ATP/GTP binding protein*.

Keywords: *Penaeus monodon*, ATP/GTP binding protein, real-time PCR

Introduction and Objective

The giant tiger shrimp (*Penaeus monodon*) is one of the economically important cultured species. Genetic improvement to increase the productivity of *P. monodon* can be carried out by applications of domestication and selective breeding program. Afterwards, advance technology can be effectively applied to promote the sustainable aquaculture of *P. monodon*.

The development of oocytes consists of a series of complex cellular events, in which different genes express to ensure the proper development of oocytes and to store transcripts and proteins as maternal factors for early embryogenesis.

To examine the possible involvement of the *ATP/GTP binding protein* in ovarian development of *P. monodon*, its full-length cDNA and expression profiles during ovarian development of intact and eyestalk-ablated *P. monodon* broodstock were examined.

Materials and Methods

2.1 Isolation of the full-length cDNA of *ATP/GTP binding protein*

The full-length cDNA of *P. monodon ATP/GTP binding protein* was obtained by further sequencing of the original EST clone at the 3′ direction using a primer walking approach.

2.2. Quantitative real-time PCR

Standard curves representing 10^3-10^8 copies of *P. monodon ATG/GTP binding protein* (F: 5' TAGCAGTGTTCACCTGGCAT 3' and R: 5' GTAGGAAAGTCAACTCTGTGC 3') and the internal control, *EF-1α* (5′-GTCTTCCCCT TCAGGACGTC-3′ and R5: 5′-CTTTACAGAC ACGTTCTTCACGTTG-3′), were constructed.

Quantitative real-time PCR of *ATP/GTP binding protein* and *EF-1α* of each shrimp was examined in duplicate. The relative expression level between shrimp possessing different stages of ovarian development were statistically tested ($P < 0.05$).

Results and Discussion

ATP/GTP binding proteins are involved in the regulation of cell growth, differentiation, and vesicular transport processes. However, the functional involvement of this gene/protein in ovarian development has not been reported in *P. monodon*.

The full-length cDNA of *P. monodon ATP/GTP binding protein* was 1547 bp in length containing an ORF of 1263 bp corresponding to a polypeptide of 420 amino acids with the 5′ and 3′UTRs of 68 and 213 bp (excluding the poly A tail; Fig. 1), respectively. It significantly matched *Pre-mRNA cleavage complex II protein Clp1* of *Harpegnathos saltator* (*E*-value = 1e-155). The calculated p*I* and MW of the deduced ATP/GTP binding protein of *P. monodon* was 5.98 and 46.52 kDa. The pre-mRNA cleavage complex II protein (Clp1) domain was found at positions 149-481 of the deduced ATP/GTP binding protein (*E*-value = 5.50e-157).

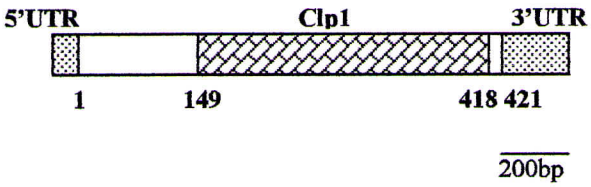

Figure 1. Schemetic diagram illustration the full-length cDNA of P. monodon ATP/GTP.binding protein. The predicted Clp1 domain is located at positions 149-418 of the deduced protein.

ATP/GTP binding protein plays an important role for the formation of the 3′ end of pre-mRNA for which the pre-mRNA is cleaved endonucleolytically. The upstream cleavage fragment subsequently polyadenylated and the downstream product degraded.

Quantitative real-time PCR revealed that the expression level of *ATP/GTP binding protein* mRNA in ovaries of both intact and eyestalk-ablated broodstock was not significantly different from that of juveniles (4-month-old shrimp) ($P > 0.05$).

In adults, the expression *P. monodon ATP/GTP binding protein* was comparable during ovarian development in both intact and eyestalk-ablated broodstock ($P > 0.05$). Interestingly, this transcript was up-regulated after spawning ($P > 0.05$).

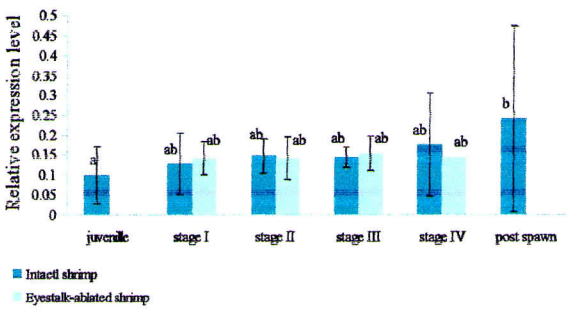

Figure 2. Relative expression profiles of P. monodon ATP/GTP binding protein during ovarian development of intact and unilateral eyestalk ablated shrimp. (N = 6 for 4-month-old juveniles, N = 8, 6, 7, 8 and 6 for stages I-IV and post-spawning broodstock and N = 3, 8, 10 and 10 for stages I – IV of eyestalk-ablated shrimp, respectively).

The information suggested that the steady state amounts of the *P. monodon ATP/GTP binding protein* mRNA may be sufficient to maintain multiple times of its translations throughout oogenesis of *P. monodon*. An increase of the *ATP/GTP binding protein* mRNA level after spawning implied that this transcript should be required for the next round of ovarian development and maturation of *P. monodon*. Therefore, the expression profiles of the ATP.GTP binding protein during ovarian development of this economically important species should be further carried out.

References

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