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The *nanos* gene of *Bombyx mori* and its expression patterns in developmental embryos and larvae tissues

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Abstract

The *nanos* gene encodes a zinc-finger protein which is required for the migration and differentiation of primordial germ cells as well as for their fate maintenance. In this study, a 1913 bp *nanos* gene was cloned and characterized in silkworm (*Bombyx mori*). RT-PCR and Western blot analysis showed that the *nanos* was expressed in developing embryos and various silkworm larval tissues. The expression patterns of Nanos and Vasa in silkworm larval gonads were analyzed using immunohistochemistry. It was found that, in silkworm larval ovaries, the Nanos and Vasa proteins were expressed in oocytes. While in testes, high expression of Nanos and Vasa was detected in spermatogonia and relatively weaker expression was found in spermatocytes at latter stages.

Keywords: *Bombyx mori*; *Nanos*; *Vasa*; Development; Primordial germ cells; RACE; Western blot; Immunohistochemistry

1. Results and discussion

In many insects, the placement and development of primordial germ cells (PGCs) have been studied using molecular markers of germ cell fate (Donnell et al., 2004). Two genes, *vasa* and *nanos*, have been proved to be useful markers for PGCs in a broad range of species. The *vasa* gene, first identified in *Drosophila melanogaster,* encodes a DEAD box RNA helicase that is expressed in the PGCs of all major groups of animals examined (Extavour, 2005; Sagawa et al., 2005). In *Drosophila*, the Vasa protein is a component of the maternal germplasm, which can be identified in germ cells in all developmental stages. Vasa expression has also been examined in *Bombyx mori* (Nakao, 1999) which revealed that Vasa protein was first expressed in the presumptive embryo and was transported to cells at the posterior germ band. In later stages, these Vasa positive cells propagated at the abdomen in regions consistent with the forming gonads. However, there has been no report on expression patterns of Vasa proteins in larval stage.

Nanos genes encode zinc-finger transcription factors. In *Drosophila*, *nanos* is required for migration of PGCs and maintenance of its germ cell fate (Kobayashi et al., 1996). Nanos proteins also act to prevent PGCs from differentiating into somatic cells (Wang and Lin, 2004). In zebrafish, *nanos* is required for migration and survival of PGCs (Koprunner et al., 2001). Mice have three *nanos*-like genes, two of which are expressed in PGCs and required for their fate maintenance (Tsuda et al. 2003).

Nanos genes play an important role in specifying posterior regions of insect embryos. In *Drosophila*, *nanos* mRNA is localized in posterior region of the embryo, and Nanos translation is repressed in other regions of the embryo by the binding of Smaug protein to an RNA secondary struc-

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ture in the 3′-UTR of the *nanos* mRNA (Dahanukar et al., 1999). Nanos protein then regulates Hunchback (Hb) translation by recruiting a cofactor, Pumilio, that binds a 'Nanos response element' in the 3′-UTR of the *Hunchback* mRNA, thus restricting Hb protein expression to the anterior of the embryo (Irish et al., 1989).

Although Nanos also plays important roles in repressing mitosis, somatic gene expression and differentiation of pole cells into somatic cells (Hayashi et al., 2004), the primary role for Nanos appears to be repressing apoptosis of germ cells. It was suggested that Nanos realizes this function through interfering the hid/skl-dependent apoptosis pathway (Tsuda et al., 2003; Hayashi et al., 2004; Sato et al., 2007). Nanos proteins were also found to be highly conserved and were required for germ-line survival (Kraemer et al., 1999) .

Nanos has been shown to express in PGCs of Diptera insect (Calvo et al., 2005), *Caenorhabditis elegans* (Schaner et al., 2003), cnidarians (Extavour et al., 2005), leech (Kang et al., 2002), mice (Tsuda et al., 2003) and humans (Jaruzelska et al., 2003). Yet there has been no report on *nanos* in *B. mori*. In this study, we cloned and characterized *nanos* homologues in *B. mori*, analyzed its expression patterns in embryos of different developmental stages and in different larval tissues. Besides, immunohistochemistry was employed to find out the Nanos and Vasa distribution in ovarial and testicular tissues of *B. mori* larvae.

1.1. Isolation and analysis of nanos from B. mori

We identified a *B. mori* mRNA sequence (Accession No. AB017535) in NCBI EST database, which has high similarity (73%) with honeybee *nanos*. Based on the mRNA sequence, we designed primers for 5′-RACE, and obtained the full-length cDNA (1913 bp) from *B. mori*. This sequence was designated as *Bm-nanos*, which has been registered in GenBank under the accession number of EF647589. Further analysis shows that the *Bm-nanos* cDNA has 7 exons and 6 introns. Its ORF is 954 bp long and is flanked by 27 bp 5′-UTR and 932 bp 3′-UTRs (Fig. 1). The putative Nanos protein has 318 amino acid residues with a molecular weight of 35 kDa. Multiple alignments of predicted proteins from Nanos homologues indicate that the highest similarity among these sequences lies in the C-terminal zinc-finger RNA-binding domain. This domain contains two CCHC motifs for coordinating zinc ions that are completely conserved in Bm-nanos (Fig. 2).

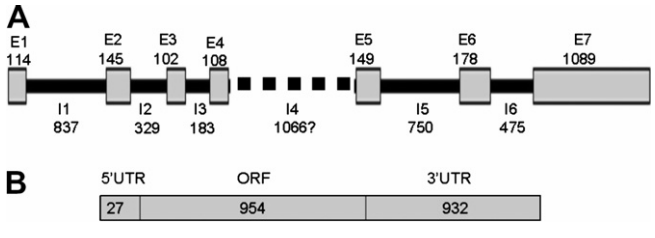

Fig. 1. (A) Schematic structure of the *Bm-nanos* gene. Numbered exons (E) are indicated as large open boxes with nucleotide lengths above the box. Introns are thick lines with nucleotide lengths below the lines. The exact length of I4 is not available since there is a gap within it. (B) The Schematic structure of the corresponding cDNA product (not to scale). The 5′-UTR, ORF and 3′-UTR of each is a box with nucleotide lengths indicated in the boxes.

Molecular and genetic analyses revealed that *nanos* mRNA localization and expression are regulated by cis-acting nucleic acid sequences in its 3′-UTR (Gavis and Lehmann, 1994; Gavis et al., 1996). Translational control of protein synthesis by specific sequences in the 3′-UTRs of their corresponding mRNAs is a feature of many transcript-specific regulatory mechanisms (Mazumder et al., 2003). An RNA structure analysis program predicts that the first 130 nt of the *Bm-nanos* 3′-UTR produces a similar RNA fold to that of the vector mosquitoes and *Drosophila* (Calvo et al., 2005; Dearden, 2006) (data not shown). In *Drosophila*, the 3′-UTR of *nanos* contains a structural RNA motif required for translational repression (Dahanukar and Wharton, 1996; Smibert et al., 1999). This secondary structure is bound by the Smaug protein in early embryos (Smibert et al., 1999). The predicted secondary structure of *Bm-nanos* mRNA in the 130nt region represents a potential protein-binding domain.

1.2. Expression profiles of Bm-nanos

To find out the timing of *Bm-nanos* RNA production, RT-PCR was carried out on cDNA from silkworm embryos of various stages. A PCR product of the target length was observed in all developmental stages (Fig. 3B). Analysis of *Bm-nanos* tissue distribution in silkworm larvae by RT-PCR revealed that the *Bm-nanos* was expressed in all tested tissues (ovary, testis, fat body, blood, midgut, silk gland, malphigian vessel and embyro) (Fig. 3A). Western blot anal-

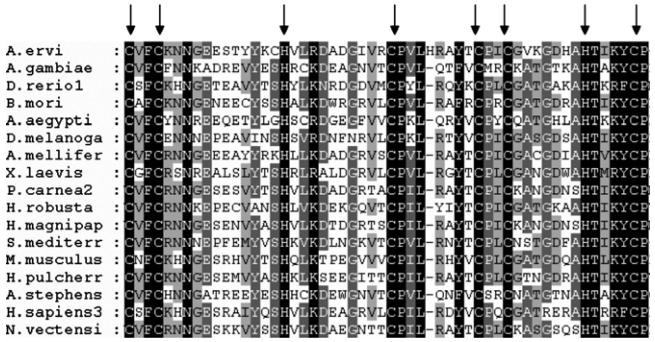

Fig. 2. Alignment of the most conserved regions of Nanos protein sequences from representatives of metazoa. The sequences were aligned using GeneDoc. The arrowheads indicate the conserved resides among Nanos proteins which can form two CCHC zinc-finger motifs. The abbreviation and their accession numbers in Swiss-Prot are: *Schmidtea mediterranea* (A4UCR6), *Mus musculus* (P60322), *Hemicentrotus pulcherrimus* (Q2WFX4), *Anopheles stephensi* (Q6PRW9), *Podocoryne carnea* (Q670V8), *Aphidius ervi* (A4ZHY7), *Homo sapiens* (P60323), *Nematostella vectensis* (Q4PLU3), *Aedes aegypti* (Q6PRX0), *Xenopus laevis* (Q07937), *Helobdella robusta* (O18467), *Hydra magnipapillata* (Q9NDN9), *Drosophila melanogaster* (P25724), *Apis mellifera* (Q2PZC1), *Anopheles gambiae* (Q6PRW8), and *Danio rerio* (Q90WW1).