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The ontogeny of *nanos* homologue expression in the oligochaete annelid *Tubifex tubifex*

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ABSTRACT

We have cloned and characterized the expression of a *nanos* homologue (designated *Ttu-nos*) from the oligochaete annelid *Tubifex tubifex*. *Ttu-nos* mRNA is distributed broadly throughout the early cleavage stages. *Ttu-nos* is expressed in most if not all of the early blastomeres, in which *Ttu-nos* RNA associates with pole plasms. *Ttu-nos* transcripts are concentrated to 2d and 4d cells. Shortly after $2d^{111}$ (derived from 2d cell) divides into a bilateral pair of NOPQ proteloblasts, *Ttu-nos* RNA vanishes from the embryo, which is soon followed by the resumption of *Ttu-nos* expression in nascent primary blast cells produced by teloblasts. The resumption of *Ttu-nos* expression occurs only in a subset of teloblast lineages (viz., M, N and Q). After *Ttu-nos* expression is retained in the germ band for a while, it disappears in anterior-to-posterior progression. At the end of embryogenesis, there is no trace of *Ttu-nos* expression. Thereafter, growing juveniles do not show any sign of *Ttu-nos* expression, either. The first sign of *Ttu-nos* expression is detected in oocytes in the ovary of young adults (ca 40 days after hatching), and its expression continues in growing oocytes that undergo yolk deposition and maturation in the ovisac.

The *nanos* (*nos*) gene encodes an RNA binding protein with two highly conserved CCHC Zn-fingers in the C terminus. This gene was first identified as a maternal effect gene in the fruit fly *Drosophila melanogaster* (Nüsslein-Volhard et al., 1987). It is now well known that in *Drosophila*, a Nos protein interacts with maternal *hunchback* mRNA and represses its translation in posterior regions of the embryo (Tautz, 1988; Irish et al., 1989). Since then, *nos*-related genes have been identified in a number of animal species (Mosquera et al., 1993; Subramaniam and Seydoux, 1999; Torras et al., 2004; Extavour et al., 2005). In some animals, functional studies have been done to demonstrate that Nos-related proteins play a pivotal role in both somatic and germline development (Kobayashi et al., 1996; Tsuda et al., 2003; Agee et al., 2006; Rabinowitz et al., 2008; Juliano et al., 2010). On the other hand, studies on embryonic expression of *nos*-related genes using whole-mount *in situ* hybridization (WISH) have also been done in a variety of animals, and suggested that irrespective of their actual function in germline cells, *nos*-related genes serve as a good marker to identify germ cells as well as to study the process of germline formation in animals.

Among lophotrochozoans, *nos*-related genes have been identified in the polychaete annelids *Platynereis dumerilli* (Rebscher et al., 2007) and *Capitella* sp. I (Dill and Seaver, 2008), the oligochaete annelid *Pristina leidyi* (Özpolat and Bely, 2015), the leech *Helobdella robusta* (Kang et al., 2002), and the snails *Haliotis asinina* (Kranz et al., 2010) and *Ilyanassa obsoleta* (Rabinowitz et al., 2008). As in other animals, germline cells such as primordial germ cells (PGCs) express *nos*-related genes in these lophotrochozoans (except for the snails mentioned above). A recent study by Cho et al. (2014) has shown that female germline cells in *Helobdella* embryo express *piwi* and *vasa* in addition to *nos*, while male PGCs express *nos* only. This *Helobdella* (hermaphrodite) study prompted us to investigate *nos* expression pattern in another hermaphrodite annelid *Tubifex tubifex*, in which unlike those in *Helobdella*, both the male and female PGCs (located in segments X and XI, respectively) are known to express a *vasa* gene at the same time during embryogenesis (Oyama and Shimizu, 2007; Kato et al., 2013).

In this study, we have isolated a *nanos* homologue from *Tubifex tubifex* and examined its expression throughout the life cycle.

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1. Results and discussion

1.1. Cloning of tubifex homologues of nanos

Using degenerate PCR and subsequent 3′ RACE, we isolated portions of two *nanos* (*nos*) homologues, *Ttu-nos1* and *Ttu-nos2*, from the oligochaete annelid *T. tubifex*. *Ttu-nos1* and *Ttu-nos2* cDNA fragments obtained were 1797 and 1794 base pairs long, respectively, and both contained a 3′ untranslated region (UTR) and two putative CCHC-type Zn-finger domains in the coding region, displaying a high degree of homology with *nos*-related proteins of other metazoans, especially lophotrochozoans (Fig. 1A and B). Both cDNAs have identical amino acid sequences (Fig. 1A) though they share 97.8% nucleotide sequence identity with each other (not shown). By contrast, 3′UTR of the cDNAs have approximately 93% identical nucleotide sequences. The DDBJ accession numbers for *Ttu-nos1* and *Ttu-nos2* are LC020234 and LC020235, respectively.

At present it is not known whether *Ttu-nos1* and *Ttu-nos2* are derived from the two different genes or result from alternative splicing of the same gene. Genomic Southern analysis would provide significant information to differentiate these possibilities.

In this study, we prepared WISH probes for both *Ttu-nos1* and *Ttu-nos2* and used them to examine the spatiotemporal distribution pattern of transcripts of these homologues. We found that both probes gave identical staining patterns in WISH preparations (data not shown). It should be mentioned, however, that embryos hybridized with a *Ttu-nos1* probe were stained more intensely than those hybridized with a *Ttu-nos2* probe even if both embryos were processed under identical conditions including RNA probe concentration. In the following sections, we will describe the expression patterns of *Ttu-nos1* only, and refer to *Ttu-nos1* as *Ttu-nos*.

1.2. Maternal Ttu-nos transcripts are distributed broadly in early embryos

Whether they are stored in the ovisac of the adult worm or

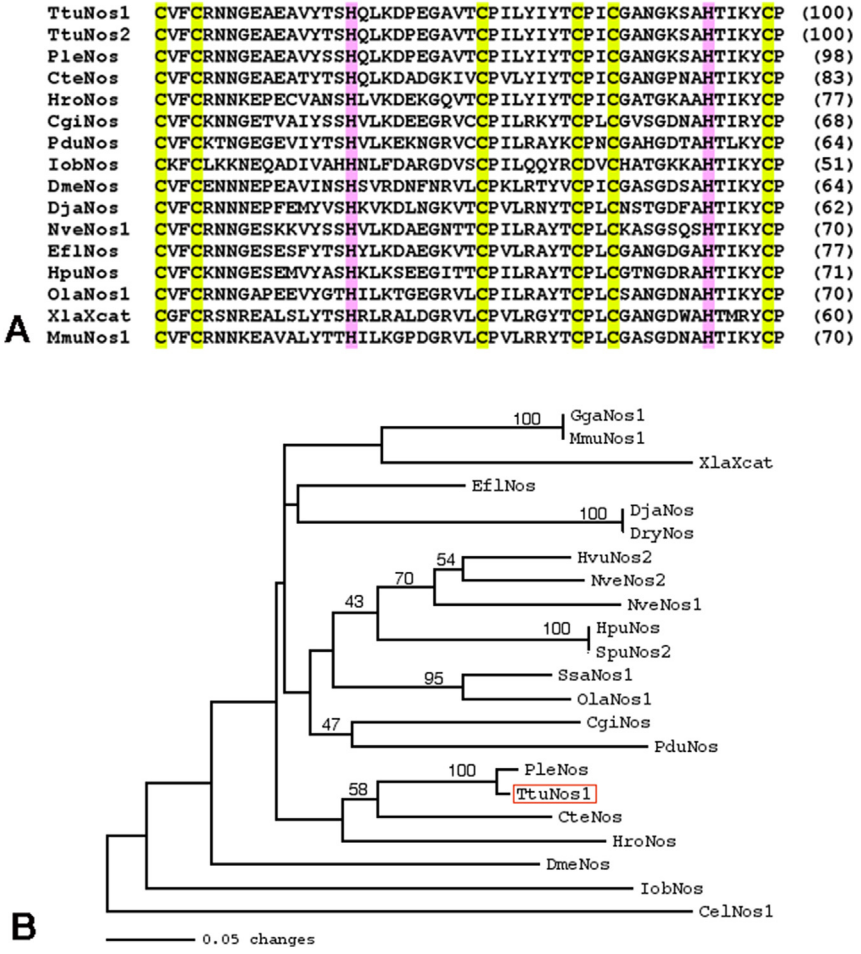

Fig. 1. Characterization of *Ttu-nos1*, a *nanos* (*nos*) homologue from *Tubifex tubifex*. (A) Alignment of the putative CCHC-type zinc finger domain of TtuNos1 with known *nos*-related proteins. Concerved C and H residues are highlighted in yellow and red, respectively. Numbers in parentheses indicate the percentage amino acid identity with TtuNos1. (B) Molecular phylogenetic analysis of the *nos* zinc finger domain. The following sequences were used: CgiNos1 (NBCI accession number EKC30400), CteNos (DAA06318), GgaNos1 (XP_003641548), DjaNos (BAD88623), DmeNos (AAA28715), DryNos (BAK57419), EflNos (BAB19253), HpuNos (BAE53723), HroNos (AAB63111), HvuNos2 (BAB01492), IobNos (ABV54788), MmuNos1 (NP848508), NveNos1 (AAW29070), NveNos2 (AAW29071), OlaNos1 (ABU63571), PduNos (CAJ28985), PleNos (ADE44350), SsaNos (NP001135057), SpuNos2 (NP001073023),*Ttu-nos*1 (LC020234),*Ttu-nos*2 (LC020235) and XlaXcat (NP001081503). The phylogenetic tree was generated by the neighbour joining method using PAUP*4.0b10. CelNos1 (NP496358) was used as an outgroup. Numbers are bootstrap values (as percentages of 1000 replications). Lengths of branches are drawn to the scale indicated. Species abbreviations: Cel *Caenorhabditis elegans* (nematode); Cgi *Crassostrea gigas* (mollusc); Cte *Capitella teleta* (annelid); Dja *Dugesia japonica* (planaria); Dme *Drosophila melanogaster* (fruit fly); Dry *Dugesia ryukyuensis* (planaria); Efl *Ephydatia fluviatilis* (sponge); Gga *Gallus gallus* (chick); Hpu *Hemicentrotus pulcherrimus* (sea urchin); Hro *Helobdella robusta* (annelid); Hvu *Hydra vulgaris* (cnidaria); Iob *Ilyanassa obsoleta* (mollusc); Mmu *Mus musculus* (mouse); Nve *Nematostella vectensis* (cnidaria); Ola *Oryzias latipes* (medaka); Pdu *Platynereis dumerilii* (annelid); Ple *Pristina leidyi* (annelid); Ssa *Salmo salar* (salmon); Spu *Strongylocentrotus purpuratus* (sea urchin); Ttu *Tubifex tubifex* (annelid); Xla *Xenopus laevis* (frog).