

Exploration of embryonic origins of germline stem cells and neoblasts in *Enchytraeus japonensis* (Oligochaeta, Annelida)

Mutsumi Sugio^{a,*}, Kazunari Takeuchi^a, Junko Kutsuna^a, Ryosuke Tadokoro^{b,c,d},
Yoshiko Takahashi^d, Chikako Yoshida-Noro^e, Shin Tochinali^a

^a Division of Biological Sciences, Graduate School of Science, Hokkaido University, N10, W8, Kita-ku, Sapporo 060-0810, Japan

^b Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine, Nagoya, Aichi 466-8550, Japan

^c Core Research for Evolutionary Science and Technology (CREST), Japan Science and Technology Corporation (JST), Kawaguchi, Saitama 332-0012, Japan

^d Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan

^e Advanced Research Institute for the Sciences and Humanities, Nihon University, Tokyo 102-0073, Japan

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Abstract

An oligochaete annelid species, *Enchytraeus japonensis*, reproduces not only asexually but also sexually. It has been reported that putative mesodermal stem cells called neoblasts contribute to blastema formation and that *Ej-piwi*⁺ germline stem cells participate in gonadal regeneration. To delineate the origin and formation of both of these stem cells, we isolated two *vasa*-related genes (*Ej-vlg1* and *Ej-vlg2*) and analyzed the expression of each along with that of germline marker gene *Ej-piwi*. In adults, *Ej-vlg1* and *Ej-vlg2* were expressed in *Ej-piwi*⁺ germline stem cells and germ cells in gonads, while only *Ej-vlg2* mRNAs were detected in neoblasts. Expression analysis during embryogenesis indicated that clusters of *Ej-vlg1*⁺/*Ej-vlg2*⁺ cells, located at the posterior ventral region in late embryos, became *Ej-vlg1*⁺/*Ej-vlg2*⁺/*Ej-piwi*⁺ germline stem cells just after embryogenesis. On the other hand, *Ej-vlg2* single positive cells with morphological characteristics of neoblasts became detectable much later after embryogenesis at the ventral position on each septum where adult neoblasts exist, although these early detected cells were much smaller in size than adult neoblasts. The present results suggest that (1) germline stem cells specified just after embryogenesis are derived from *Ej-vlg1*⁺/*Ej-vlg2*⁺ cells which appear at the posterior ventral region in late embryos, and that (2) neoblasts appear much later in development.

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Keywords: Germline; Germline stem cell; Stem cell; Neoblast; Growth zone; Germ cell; *Vasa*-related gene; *Ej-vlg1*; *Ej-vlg2*; *Ej-piwi*; Embryogenesis; Gonadal regeneration; *Enchytraeus japonensis*; Earthworm; Oligochaete; Annelida

1. Results and discussion

An enchytraeid worm, *Enchytraeus japonensis*, spontaneously autotomizes into several fragments, and each fragment regenerates into a complete new worm, resulting in asexual propagation. On the other hand, under a low population density, it occasionally regenerates hermaphroditic gonads and undergoes sexual reproduction (Myohara et al., 1999). It has been reported that proposed stem cells

called neoblasts contribute to the mesodermal region of blastema and that *Ej-piwi*⁺ germline stem cells participate in gonadal regeneration. It is also suggested that the germ and somatic lineages are segregated in asexually growing animals and behave differently during regeneration (Tadokoro et al., 2006).

In order to delineate the embryonic origin of germ line cells supporting gonadal regeneration in adult *E. japonensis*, we explore the origin and the formation of germline stem cells along with neoblasts. Because *vasa*-related genes are not only expressed in germline cells but also in pluripotent stem cells in hydras (Mochizuki

* Corresponding author. Tel./fax: +81 11 706 4464.

E-mail address: mutumi@bio.sci.hokudai.ac.jp (M. Sugio).

et al., 2001), we have isolated two *vasa*-like genes (*Ej-vlg1* and *Ej-vlg2*) in the expectation of obtaining a marker for germ cells and/or neoblasts, and analyze the expression patterns of these genes along with that of *Ej-piwi*.

1.1. Isolation and characterization of *E. japonensis vasa*-related genes

We amplified DEAD-box fragments from the complementary DNA (cDNA) library of mature sexual worms

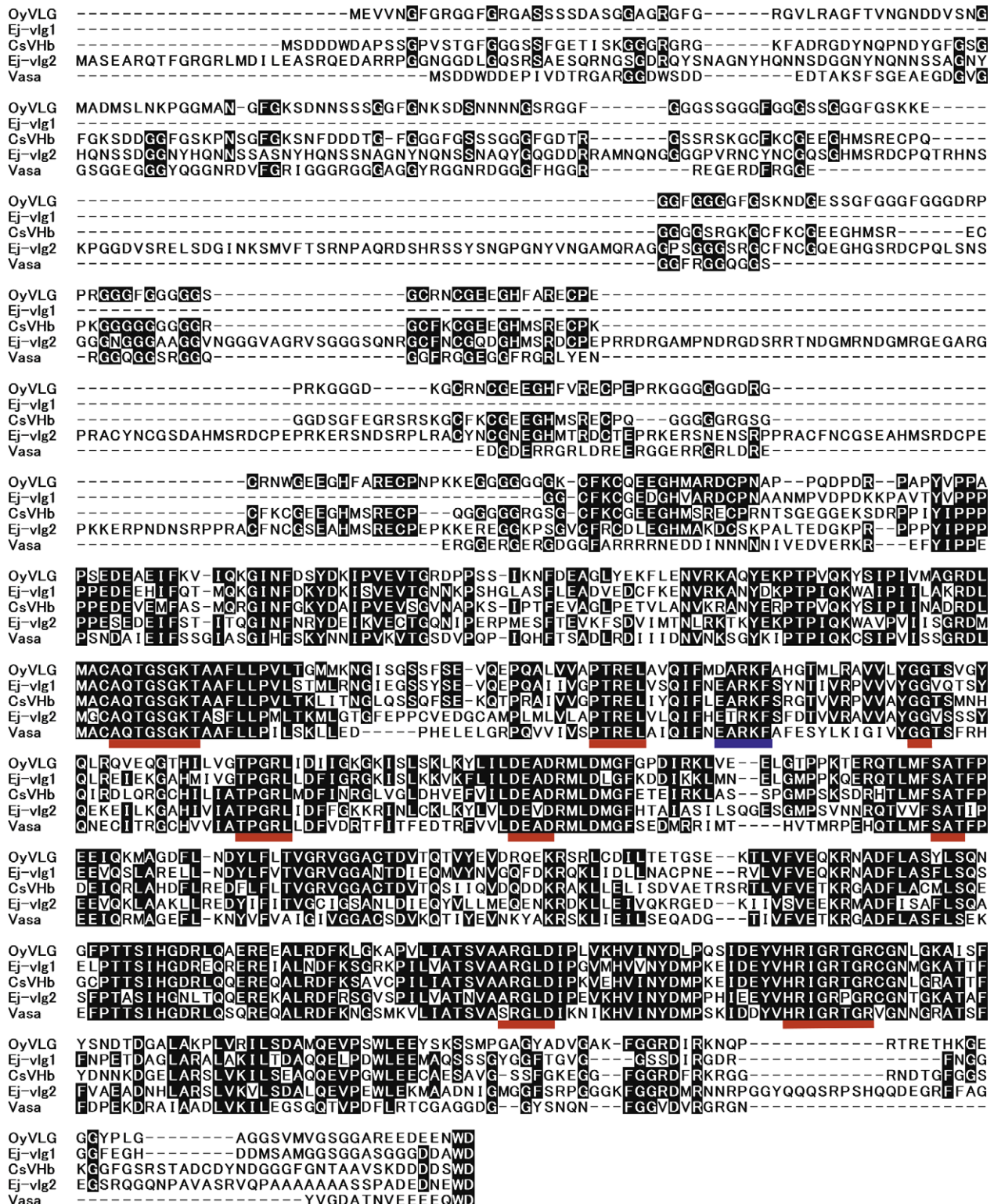


Fig. 1. Alignment of predicted amino acid sequences of *Ej-vlg1*, *Ej-vlg2*, *Vasa* (fly), *OyVLG* (oyster), and *CsVHb* (solitary ascidian). Eight DEAD-box motifs (red underline) and the EXRKF motif (blue underline), the latter of which is specifically conserved in *Vasa*- and *PL10*-related proteins, were included in both sequences. Multiple sequence alignment was performed with ClustalW software (version 1.83, default parameters).

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