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Primordial germ cells in an oligochaete annelid are specified according to the birth rank order in the mesodermal teloblast lineage

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ABSTRACT

The primordial germ cells (PGCs) in the oligochaete annelid *Tubifex tubifex* are descentants of the mesodermal (M) teloblast and are located in the two midbody segments X and XI in which they serve as germline precursors forming the testicular gonad and the ovarian gonad, respectively. During embryogenesis, *vasa*-expressing cells (termed presumptive PGCs or pre-PGCs) emerge in a variable set of midbody segments including the genital segments (X and XI); at the end of embryogenesis, pre-PGCs are confined to the genital segments, where they become PGCs in the juvenile. Here, using live imaging of pre-PGCs, we have demonstrated that during *Tubifex* embryogenesis, pre-PGCs (defined by Vasa expression) stay in segments where they have emerged, suggesting that it is unlikely that pre-PGCs move intersegmentally during embryogenesis. Thus, it is apparent that pre-PGCs derived from the 10th and 11th M teloblast-derived primary m blast cells (designated m10 and m11) that give rise, respectively, to segments X and XI are specified in situ as PGCs and that those born in other segments become undetectable at the end of embryogenesis. To address the mechanisms for this segment-specific development of PGCs, we have performed a set of cell-transplantation experiments as well as cell-ablation experiments. When m10 and m11 that are normally located in the mid region of the embryo were placed in positions near the anterior end of the host embryo, these cells formed two consecutive segments, which exhibited Vasa-positive PGC-like cells at early juvenile stage. This suggests that in terms of PGC generation, the fates of m10 and m11 remain unchanged even if they are placed in ectopic positions along the anteroposterior axis. Nor was the fate of m10 and m11 changed even if mesodermal blast cell chains preceding or succeeding m10 and m11 were absent. In a previous study, it was shown that PGC development in segments X and XI occurs normally in the absence of the overlying ectoderm. All this strongly suggests that irrespective of their surrounding cellular environments, m10 and m11 autonomously generate PGCs. We propose that m10 and m11 are exclusively specified as precursors of PGCs at the time of their birth from the M teloblast and that the M teloblast possesses a developmental program through which the sequence of mesodermal blast cell identities is determined.

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Introduction

Primordial germ cells (PGCs), the precursor stem cells to the germline, are usually established early during embryonic development. The specification of PGCs that precedes the generation of germ cells is a crucial event for all sexually reproducing animals. The modes of PGC specification have been divided into two categories, preformation and epigenesis (Extavour and Akam, 2003). In the preformation mode, the localization of maternally inherited determinants is involved in PGC specification as seen in the fruit fly *Drosophila melanogaster* (Illmensee and Mahowald, 1974; Williamson and Lehmann, 1996), the nematode *Caenorhabditis elegans* (Deppe et al., 1978; Kimble and White, 1981; Strome and Wood, 1982), zebrafish *Danio rerio* (Yoon et al., 1997) and the frog *Xenopus laevis* (Tanabe and Kotani, 1974; Züst and Dixon, 1975; Ikenishi et al., 1986). In contrast, the epigenetic mode of PGC specification depends on inductive signals from surrounding tissues as seen in mice (Ying and Zhao, 2001; Tsang et al., 2001; Saitou et al., 2002) and urodele amphibians (Nieuwkoop, 1947; Sutasurya and Nieuwkoop, 1974). Recently, however, it has been reported that small micromeres of 32-cell embryo of sea urchins, which normally contribute to the germline, are specified

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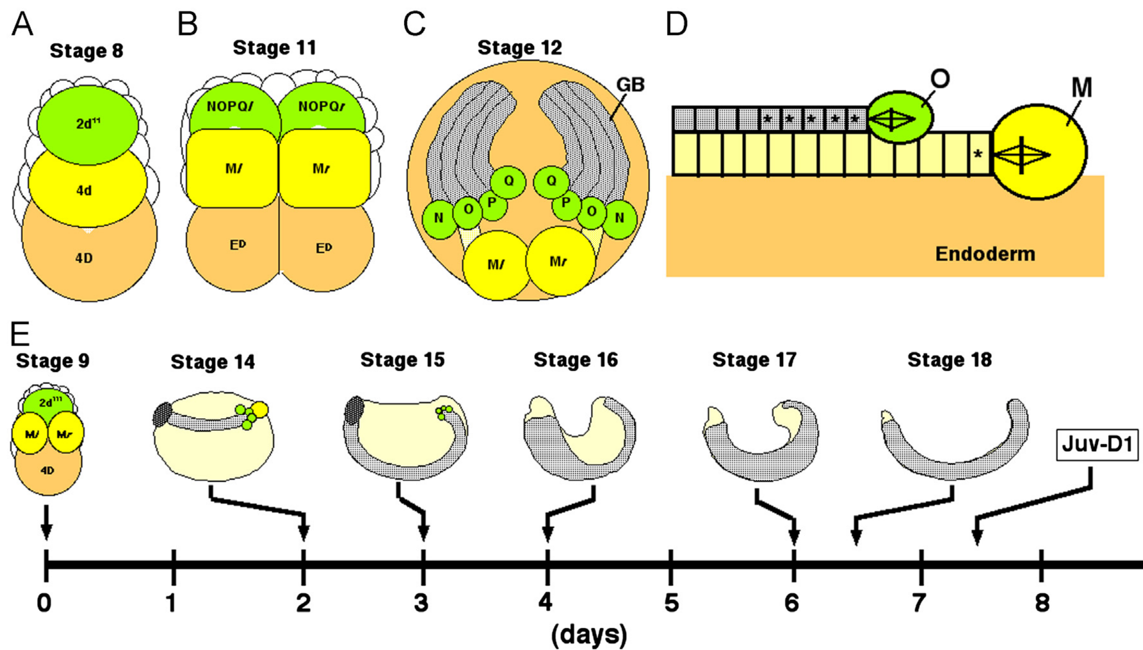

Fig. 1. Summary of *Tubifex* development. (A) A stage 8 embryo comprised of 24 cells, dorsoposterior view. Cells $2d^{11}$, 4d and 4D (sister cell of 4d) all come to lie in the future midline of the embryo. (B) A stage 11 embryo shortly after formation of mesodermal teloblasts *Ml* and *Mr* (yellow), ectodermal teloblast precursors NOPQ*l* and NOPQ*r* (green), and endodermal precursors E^D (brown). (C) A stage 12 embryo undergoing germ band (GB) formation, dorsal view with anterior to the top. (D) Longitudinal section showing the relative positions of the endoderm and bandlets extending from teloblasts M and O. Anterior is to the left and posterior is to the right. In both teloblasts, metaphase mitotic spindles are depicted to indicate the direction and inequality of their divisions. Asterisks indicate the presence of a single primary blast cell in each block of the bandlet; the remaining blocks individually represent a cell cluster, which is derived from a single primary blast cell. (E) Time course of *Tubifex* development (stages 9 to Juv-D1 or one-day-old juvenile, at 22 °C). A stage 9 embryo completes 4d cell division into a pair of teloblasts *Ml* and *Mr*. Embryos at stages 14–18 are viewed from side with anterior to the left and dorsal to the top. The GBs (hatched) are initially located on the dorsal side of the embryo (stage 14). Along with their elongation, they gradually curve round toward the ventral midline and finally coalesce with each other along the ventral midline (stage 15). The coalescence is soon followed by dorsalward expansion of the GBs (stage 16). The edges of the expanding GBs on both sides of the embryo finally meet along the dorsal midline to enclose the yolky endodermal tube (stages 16–18). Concurrently with this enclosure, the embryo becomes elongated in an anterior-to-posterior progression, and curved with the ventral convexity (stages 16–18). Enclosed portions of the embryo begin to exhibit peristaltic movements. Embryogenesis is judged to be complete when the expanding GBs have enclosed the posterior end of the embryo (stage 18), which then exhibits movement throughout its length.

autonomously as precursors of PGCs even though these cells do not inherit obvious preformed germ line components (Juliano et al., 2006; Yajima and Wessel, 2011, 2012); it is apparent that some non-epigenetic mechanism for PGC specification must operate in sea urchins. This may suggest that mechanisms for PGC specification operating in metazoans are more variable than has been thought.

In this paper, we address the mechanism for PGC specification in an oligochaete annelid *Tubifex tubifex*. This animal is a hermaphrodite with a pair of testes in segment X and a pair of ovaries in segment XI (Dixon, 1915). Germ cells in these genital segments have been thought to originate from PGCs that are located therein around the time of completion of embryogenesis (Goto et al., 1999a; also see Shimizu, 1982 for review). A previous study has shown that during *Tubifex* embryogenesis, presumptive PGCs (pre-PGCs defined by *vasa* expression) emerge in a variable set of midbody segments including the genital segments (X and XI) and that nearly all of the *vasa*-expressing cells (i.e., pre-PGCs) but those in segments X and XI become undetectable by the end of embryogenesis (Oyama and Shimizu, 2007). In newborn juveniles, thus, *vasa*-expressing cells (now designated PGCs) are confined to segments X and XI.

As to the embryonic origin of *Tubifex* PGCs in juveniles, it is possible that pre-PGCs born in segments X and XI could be specified in situ as PGCs. Alternatively, it is equally possible that pre-PGCs that have migrated to these two segments from elsewhere could become PGCs therein, since migration of PGCs in embryos and larva has often been observed in a variety of animals including a polychaete annelid (Shinomiya et al., 2000; Kobayashi et al., 2005; Rebscher et al., 2007; Yajima and Wessel, 2012). To differentiate these possibilities, it is prerequisite to have information about the behavior of pre-PGCs in living embryos, because the number and the position (along the longitudinal body axis or AP axis) of pre-PGCs are highly variable among embryos and because our previous study (Oyama and Shimizu, 2007) that was based on the observations on fixed specimens failed to provide conclusive information on this issue.

The present study was undertaken to gain an insight into the mechanism for segment-specific PGC development in *T. tubifex*. The objectives of this study were (i) to determine the origin of pre-PGCs that are located in segments X and XI around the time of completion of embryogenesis and (ii) to examine whether the segment-specific PGC development depends on external cues. For this purpose, we developed a method that allows us to visualize pre-PGCs in living embryos. Furthermore, we utilized embryological techniques such as cell ablation and cell transplantation in combination with immunostaining of a germline marker protein Vasa. The results reported herein show that pre-PGCs born in segments X and XI are specified in situ as PGCs whereas those born in other segments become undetectable at the end of embryogenesis and that PGC specification in segments X and XI occurs independently of the interactions with surrounding tissues and the positional cues residing in the embryo. On the basis of these findings, we suggest that PGCs in *T. tubifex* are specified according to their genealogical position in the mesodermal teloblast lineage.

Materials and methods

Embryos

Embryos of the freshwater oligochaete *T. tubifex* Müller were obtained as previously described (Shimizu, 1982) and cultured at

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