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Embryonic expression of *festina lente* (*fel*), a novel maternal gene, in the oligochaete annelid *Tubifex tubifex*

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ABSTRACT

We have cloned and characterized the expression of a novel maternal gene *festina lente* (designated *Ttu-fel*) from the clitellate annelid *Tubifex tubifex*. Northern blot analyses have shown that *Ttu-fel* mRNA is approximately 8 kbp in length and that its expression is restricted to oocytes undergoing maturation division and early embryos up to 22-cell stage. Maternal transcripts of *Ttu-fel* are first detected in oocytes in the ovary of young adults (ca. 40 days after hatching); its expression continues in growing oocytes in the ovisac. *Ttu-fel* mRNA is distributed broadly throughout the egg undergoing maturation divisions. During the process of ooplasmic segregation that results in the pole plasm formation, *Ttu-fel* mRNA becomes concentrated to the animal and vegetal poles. The RNA in the animal hemisphere is distributed in a gradient with highest concentration in the cortical region. During the first two cleavages, *Ttu-fel* mRNA is segregated to CD cell then to D cell; it is subsequently inherited by the three D quadrant micromeres, 1d, 2d and 3d. Around the time of transition to 22-cell stage, *Ttu-fel* mRNA becomes undetectable throughout the embryo.

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Early development in the animal embryo is driven by maternally provided gene products such as mRNAs and proteins, though at some point during development there is a transition to control by zygotic gene products (Davidson, 1986). Recent studies on model organisms such as nematode, fruit fly and mouse have demonstrated that the first event of the maternal-to-zygotic transition (MZT) is the elimination of maternal transcripts and that 30–35% of maternal mRNAs are destabilized during the period of this transition in these animals (Schier, 2007; Tadros and Lipshitz, 2009). Given that the MZT occurs in all animal embryos, it is highly possible that similar maternal mRNA elimination takes place during early development in all metazoans.

Although there have been few systematic studies on the events occurring during the MZT in spiralian lophotrochozoans, it has been known that in the clitellate annelid (leech) *Helobdella*, zygotic transcripts are needed for early cleavages (Bissen and Weisblat, 1991; Bissen and Smith, 1996; Schmerer et al., 2013). Similar early requirement for zygotically produced mRNAs has recently been suggested in unequal cleavage of the teloblast precursor cell 2d in another clitellate annelid (oligochaete) *Tubifex* (M. Aoki and T. Shimizu, unpublished data). On the other hand, in these annelids, not a few maternal genes have been cloned and examined for their expression patterns, including *Tubifex* (*Ttu*) homologues of *dorsal* (*dl*), *nanos* (*nos*), *p68* and *vasa* (*vas*) (Matsuo et al., 2005; Oyama and Shimizu, 2007; Oyama et al., 2008; Mohri et al., 2016) and *Helobdella* (*He*) homologues of *hunchback*, *msx*, *nos*, *piwi*, *twist*, *vas* and *wnt* (Kostriken and Weisblat, 1992; Master et al., 1996; Savage and Shankland, 1996; Soto et al., 1997; Kang et al., 2002; Cho et al., 2014). Semi-quantitative analyses have shown that maternally supplied transcripts of *Ttu-nos*, *He-nos*, *He-vas* and *He-piwi* decrease in amount during early cleavage stages and that this decrease is followed by upregulation of their expression that occurs during mid development (Kang et al., 2002; Cho et al., 2014; Mohri et al., 2016). In contrast, maternal transcripts of *Ttu-p68* and *Ttu-vas* seem not to decrease in amount significantly during early and mid development, but do so during late development (Oyama and Shimizu, 2007; Oyama et al., 2008).

In this study, we have isolated a novel maternal gene named *festina lente* (*fel*) from *Tubifex tubifex*. Compared with other maternal genes described so far, this gene is apparently unique in

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that its maternal mRNA is detected exclusively during the period of early cleavage stages and that it is inherited by the second micromere (2d) but not by the fourth micromere (4d) of the D quadrant.

1. Results and discussion

1.1. Summary of early development of T. tubife

A brief review of *Tubifex* development is presented here as a background for the observations described below (for details, see Shimizu, 1982). *Tubifex* zygotes, which are oviposited at metaphase I, form two polar bodies and then enter the first mitosis. Before the first cleavage, yolk-deficient cytoplasm called pole plasm accumulates at both poles of the egg (Fig. 1A). The early development of *Tubifex* consists of a stereotyped sequence of cell divisions. The first cleavage of the *Tubifex* egg is unequal and meridional, and produces a smaller AB cell and a larger CD cell (Fig. 1B). The second cleavage is also meridional and yields cells A, B, C and D: the CD cell divides into a smaller C cell and a larger D cell while the AB cell separates into cells A and B of various sizes (Fig. 1C). From the third cleavage on, the quadrants A, B and C repeat unequal divisions three times, and the D quadrant four times, producing micromeres at the animal side and macromeres at the vegetal side. The D quadrant micromeres are designated 1d, 2d, 3d and 4d (Fig. 1D–G). Cells 2d and 4d are much larger than cells 1d and 3d. During cleavages, the pole plasms are inherited by the D lineage cells and finally partitioned into 2d and 4d. At 22-cell stage, $2d^{11}$ (derived from 2d), 4d and 4D (sister cell of 4d) all come to lie in the future midline of the embryo (Fig. 1G). These three cells undergo bilateral cell division in the next cleavage: 4d divides equally yielding the left and right mesoteloblasts M (Fig. 1H); 4D divides equally into endodermal precursors E (Fig. 1I); and $2d^{111}$ (derived from $2d^{11}$) divides into a bilateral pair of ectoteloblast precursors NOPQ (Fig. 1J). The quadrant cells A, B and C also divide equally at the sixth cleavage.

1.2. Cloning of a "2d-cell-specific" gene, festina lente, from T. tubifex

During the course of preliminary whole-mount *in situ* hybridization (WISH) using riboprobes that had been synthesized from 'positive' clones resulting from screening of a 1-cell-stage cDNA library (see Experimental procedures), we found that a riboprobe synthesized from clone 4038 stained 2d cell but not 4d cell of a 22-cell embryo. (Those from the remaining clones stained both 2d and 4d of the same embryos.) This preliminary observation suggests that clone 4038 contains a *Tubifex* gene whose transcripts are segregated preferentially to 2d cell. As described later, this gene is a novel maternal gene from spiralian lophotrochozoans, and its expression is restricted to early cleavage stage embryos. Hereafter we will name this gene *festina lente* (*fel*).

Clone 4038 was found to contain a 1852-bp cDNA sequence which includes a 40-bp poly-A tail at the 3′ end (DDBJ/GenBank accession number LC140925). To examine whether the 1812-bp sequence represents full length of *Ttu-fel* transcripts, we performed a Northern blot analysis using RNA isolated from various stages of *Tubifex* development. We found that "intact" *Ttu-fel* transcripts were approximately 8 kbp in length (Fig. 2). This

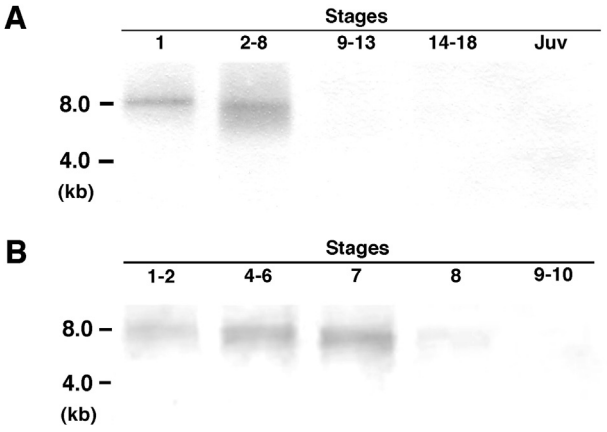

Fig. 2. Expression of *Ttu-fel* mRNA. Northern blot analysis of total RNA (10 μg each) of (A) embryos at stages 1, 2–8, 9–13, 14–18, and Juv (10-day-old juveniles) or (B) embryos at stages 1–2, 4–6, 7, 8, and 9–10. Total RNA was prepared and electrophoresed as described in Experimental procedures. The membrane was probed with DIG-labeled RNA. For stages 1–11, see Fig. 1. Stage 12, ectodermal teloblastogenesis; stages 13–15, germ band formation; stages 16–18, body elongation accompanied by formation of segmental ectoderm and mesoderm (for details of developmental stages, see Shimizu, 1982).

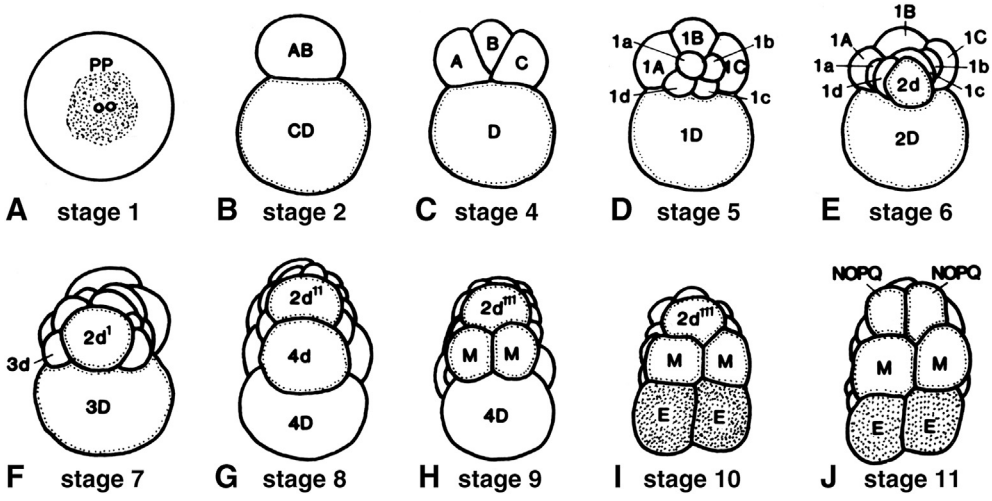

Fig. 1. Summary of *Tubifex* early development. Diagrammatic illustration of stages 1–11 of embryonic development (stage 3 is omitted). (A–C) Animal pole view of embryos at stages 1-cell (A), 2-cell (B) and 4-cell (C). pp, pole plasm. (D–G) Animal pole view of embryos undergoing the formation of the D quadrant micromeres 1d (D), 2d (E), 3d (F) and 4d (G). (H–J) Posterior view of embryos undergoing bilateral cell division in $2d^{111}$ (derived from $2d^{11}$), 4d and 4D. (H) 4d divides into a pair of mesoteloblasts (M). (I) 4D divides into a pair of endodermal precursors (E). (J) $2d^{111}$ divides into a pair of ectoteloblast precursors (NOPQ) (Redrawn from Shimizu, 1982).

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