DIVISION OF DEVELOPMENTAL GENETICS

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Germ cells are the specialized cells that can transmit the genetic materials from one generation to the next in sexual reproduction. All the other cells of the body are somatic cells. This separation of germ and somatic cells is one of the oldest problems in developmental biology. In many animal groups, a specialized portion of egg cytoplasm, or germ plasm, is inherited by the cell lineage which gives rise to germ cells. This cell lineage is called germline. The germline progenitors eventually migrate into the gonads, where they differentiate as germ cells when the organisms are physically matured. Earlier investigators have demonstrated that germ plasm contains maternal factors required and sufficient for germline formation. In *Drosophila*, this cytoplasm is histologically marked by the presence of polar granules, which act as a repository for the maternal factor required for germline formation. Our molecular screens have identified several factors stored in the polar granules.

I. The role of mitochondrial ribosomal RNAs in pole cell formation

Ultrastructural studies have shown that the germ plasm is basically composed of polar granules and mitochondria. While the primary roles of the mitochondria are oxidative phosphorylation and biosynthesis of many metabolites, it has now become evident that they are also involved in the formation of the germline progenitors, or pole cells. In *Drosophila*, pole cell formation requires the function of mitochondrial ribosomal RNA in germ plasm. We have previously reported that mitochondrial large rRNA (mtlrRNA) and small rRNA (mtsrRNA) are both transported from mitochondria to polar granules. This transportation occurs during early embryogenesis, when mitochondria are tightly associated with polar granules in germ plasm. Mitochondrial rRNAs remain on the polar granules until pole cell formation and are no longer discernible on the granules within pole cells. Reduction of the extra-mitochondrial mtlrRNA amount results in the failure to form pole cells and injection of mtlrRNA is able to induce pole cells in embryos whose ability to form these cells has been abolished by uv-irradiation. These observations clearly show that the extra-mitochondrial mtlrRNA on polar granules has an essential role in pole cell formation, cooperating with mtsrRNA (Figure 1).

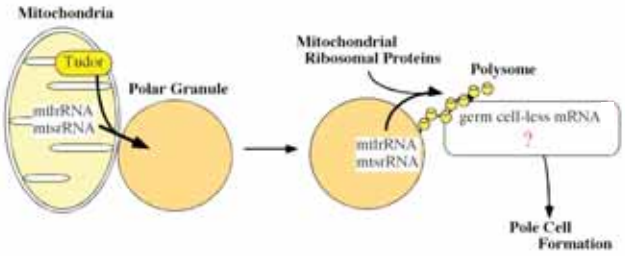

Figure 1. Role of mitochondrial rRNAs in pole cell formation

Recently, we have found that injection of kasugamycin (KA) and chloramphenicol (CH), inhibitors for prokaryotic-type translation, disrupt pole cell formation in early embryos. The number of mitochondrial-type ribosomes on polar granules is significantly decreased by KA treatment, as shown by electron microscopy (Figure 2). In contrast, ribosomes in the mitochondria and mitochondrial activity are unaffected by KA and CH. Furthermore, injection of KA and CH impairs production of Germ cell-less (Gcl) protein, which is required for pole cell formation. The above observations suggest that mitochondrial-type translation is required for pole cell formation, and Gcl is a probable candidate for the protein produced by this translation system.

II. The role of maternal Nanos protein

Among the maternal components of germ plasm, Nanos (Nos) is essential for the germline-specific events occurring in pole cells. Nos mRNA is localized in the germ plasm during oogenesis, and is translated in situ to produce Nos protein after fertilization. Nos is only transiently present in the posterior half of embryos during the preblastoderm stage, and is required there for posterior somatic patterning. Nos in the germ plasm is more stably inherited into the pole cells at the blastoderm stage, remaining detectable in these cells throughout embryogenesis. Pole cells that lack Nos (*nos*$^-$ pole cells) are unable to follow normal germline development; they fail to migrate properly into the embryonic gonads. Nos represses translation of mRNAs with discrete RNA sequences called Nos response elements (NREs). In the pathway leading to posterior somatic patterning, Nos acts together with unlocalized Pumilio (Pum) protein to repress translation of maternal *hunchback* (*hb*) mRNA. This translational repression is mediated by binding of Pum to NREs in the 3'-untranslated region (UTR) of hb mRNA. In pole cells, Nos also acts with Pum to regulate germline-specific events. Pum, like Nos, is required in pole cells for their migration to the gonads.

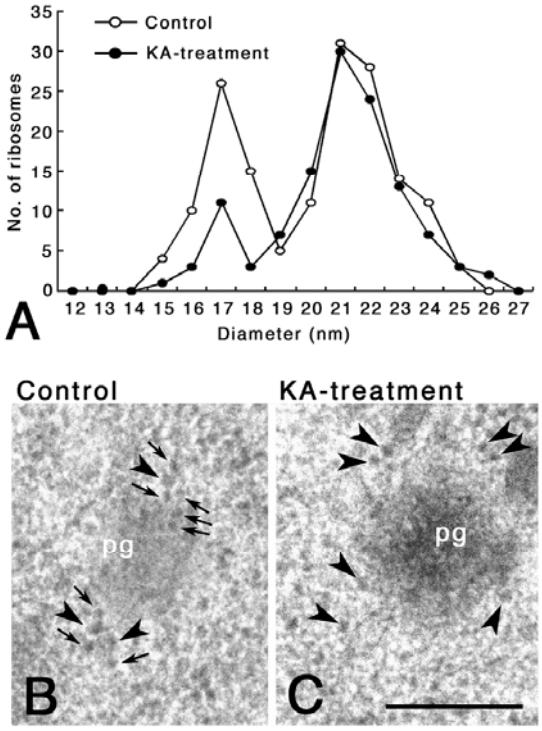

Figure 2. Injection of KA reduces the number of mitochondrial-type ribosomes around polar granules. A, The average number of ribosomes in an unit area (8.4×10^3 nm^2) is plotted against their diameter. The diameters of ribosomes around polar granules were measured in stage 2 embryos injected with DW (control) and KA (KA-treatment). The number of ribosomes smaller than 20 nm in diameter was significantly decreased by KA treatment. B, C, Electron micrographs of sections through polar granules in stage 2 embryos. B, In a control embryo injected with DW, there were two types of ribosomes around the polar granules. C, In an embryo injected with KA, the smaller ribosome was indiscernible around polar granules. Arrows and arrowheads point to the smaller (mitochondrial-type) and the larger (cytosolic-type) ribosomes, respectively. pg, polar granules; Scale bar = 0.2 μm.

Our unpublished observations suggest that one of the targets of Nos and Pum is *head involution defective* (*hid*) mRNA, which also contains an NRE in its 3'UTR and encodes a protein required for the induction of apoptosis. In the absence of Nos or Pum, migrating pole cells are eliminated by an apoptotic mechanism which is initiated at stage 9/10 in the developing embryo (Figure 3). We have also found that *Df(3L)H99* (H99), a small deletion within the genomic region that includes the *hid* gene, suppresses apoptosis in *nos* pole cells. In embryos lacking both maternal Nos and zygotic H99 activity (*nos*-H99 embryo), there is no apoptotic death of any pole cells. In addition, *nos*-H99 pole cells have the ability to migrate into the gonads when transplanted into normal host embryos. Therefore, the ability of *nos* pole cells to migrate into the gonads is fully restored by the suppression of apoptosis in our transplantation experiments. This clearly demonstrates that Nos inhibits the apoptotic response in pole cells to permit their proper migration into the gonads.

The above observations suggest that pole cells have the potential to enter into apoptosis, which somewhat contradicts the notion that the germline is fundamentally immortal as it is required for the propagation of any given species. We speculate, however, that this apoptotic pathway may be part of a mechanism that eliminates "aberrant pole cells" that have inherited an insufficient quantity of germ plasm components, such as maternal Nos protein.

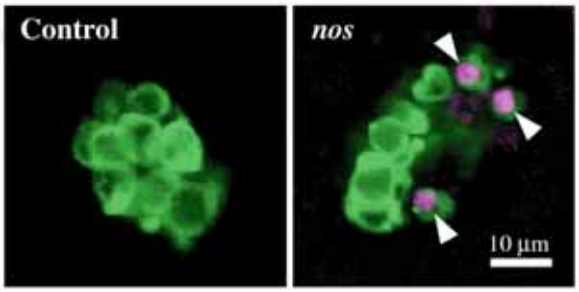

Figure 3. Nanos prevents apoptosis in pole cells. Confocal images of the pole cells in control (left) and *nos* (right) embryos at stage 13, stained with TUNEL labeling (magenta) and an antibody against Vas (green). Arrowheads show TUNEL-positive pole cells.

III. The role of maternal Sva53 in meiosis

Meiosis is an obligatory step to produce haploid gametes that can transmit the genetic materials from one generation to the next. However, little is known about how the germline progenitors acquire the ability to undergo meiosis. We have found that a novel maternal factor, SVA53 is essential for meiosis. SVA53 is a member of widely conserved BTB/POZ-zinc finger proteins, which are known to function as transcriptional regulators by altering chromatin structures. Maternal SVA53 is enriched in pole cells, and a reduction in its activity prevents meiosis. We propose that SVA53 is the first maternal molecule that regulates a genetic pathway leading to meiosis. Thus, our current findings provide the basis for the understanding of a novel epigenetic mechanism that regulates the meiotic cell cycle in *Drosophila* and in a variety of other animal groups.

IV. Expression of meiotic genes in pole cells

In *Drosophila*, genetic screens have identified many genes required for meiotic division. However, it remains elusive as to when and how these meiotic genes are activated during germline development. To obtain insights into their regulatory mechanisms, we examined the expression of 38 meiotic genes in pole cells during embryogenesis. We have found that the transcripts of 12 meiotic genes were enriched in pole cells within the embryonic gonads. Among them, *bag of marbles* (*bam*), *benign gonial cell neoplasia* (*bgcn*), *deadhead* (*dhd*), *matotopetli* (*topi*) and *twine* (*twe*) were activated only in

pole cells within the gonads, whereas the transcripts from *grapes* (*grp*), *Kinesin-like protein at 3A* (*Klp3A*), *pavarotti* (*pav*), *lesswright* (*lwr*), *mei-P26*, *Topoisomerase 2* (*Top2*) and *out at first* (*oaf*) were distributed ubiquitously in early embryos and then became restricted to pole cells and to a subset of somatic tissues at later embryonic stages. These observations suggest that pole cells have already acquired the potential to express several meiotic genes. Our data will thus provide a useful basis for analyzing how the germline acquires a potential to execute meiosis.

V. Microarray analysis of pole cells

To explore the regulatory mechanism of germline specification, we attempted to identify genes expressed in pole cells during embryogenesis. From the embryos carrying EGFP-vasa transgene that express GFP only in pole cells, we isolated pole cells by using fluorescence-activated cell sorting (FACS), and they were used for hybridization of microarray that contains probes for all predicted genes in *Drosophila* genome. Our microarray analysis has identified approximately 300 maternal and 200 zygotic transcripts enriched in polar plasm and pole cells. The functional analysis of these transcripts is now ongoing.

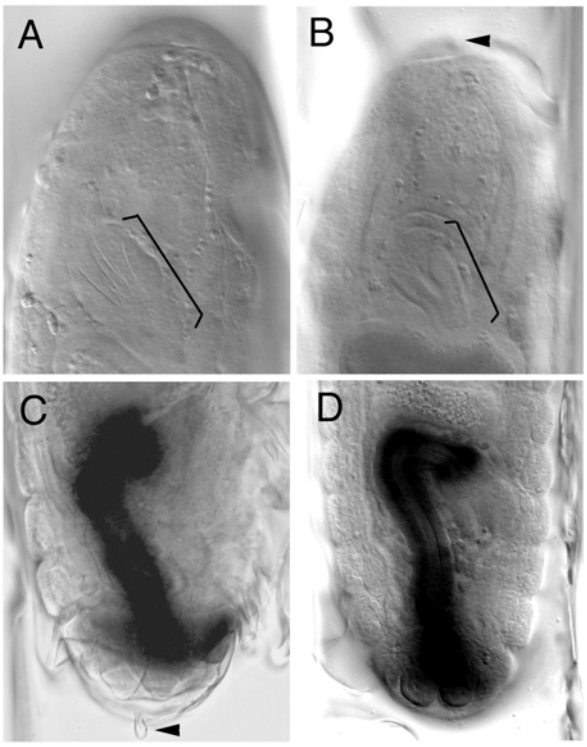

Figure 4. LR asymmetries of the proventriculus and the hindgut in embryos with fully reversed AP polarities. Dorsal views of the anterior (C, B) and the posterior region (A, D) of normal embryos (B, D) and A/P-reversed embryos (A, C) at stage 15-16. A, B, Brackets mark the proventriculi. Note that the posterior of the proventriculi are oriented to the right side of the embryos. C, D, The hindguts are stained. The anterior of the hindguts are oriented to the right. Arrowheads point to the micropyles, a morphological marker for the anterior pole of the eggs.

VI. Left-right asymmetry in embryos

In many animal groups, left-right (LR) asymmetry within the body is observed. The left and right sides of the body are generally defined with reference to the anterior-posterior (AP) and dorsal-ventral (DV) axes. We investigated whether LR asymmetry is solely dependent on the AP and DV polarities in *Drosophila* embryos. We focused on the proventriculus, a posterior part of the foregut, and the hindgut because LR asymmetry in these body parts is highly stable in normal embryos. In embryos with a fully reversed AP polarity, LR asymmetry in both the proventriculus and the hindgut was re-oriented in relation to the reversed AP polarity (Figure 4). This demonstrates that inversion of AP polarity does not affect LR asymmetry of these tissues, and implies that LR asymmetry is specified in relation to the AP and DV polarities. Our findings were not consistent with the alternative hypothesis that LR asymmetry is pre-determined by maternal signals that localize asymmetrically along the LR axis in the oocyte and/or early embryo. This work was supported by the Super Science High school (SSH) program.

Publication List:

Original papers

Amikura, R., Sato, K., and Kobayashi, S. (2005). Role of mitochondrial ribosome-dependent translation in germline formation in *Drosophila* embryos. Mech. Dev. *122*, 1087-1093.

Hayashi, M., Aono, H., Ishihara, J., Oshima, S., Yamamoto, H., Nakazato, Y., and Kobayashi, S. (2005). Left-right asymmetry in the alimentary canal of the *Drosophila* embryo. Develop. Growth Differ. *47*, 457-460.

Mukai, M., Kitadate, Y., Arita, K., Shigenobu, S., and Kobayashi, S. (2005). Expression of meiotic genes in the germline progenitors of *Drosophila* embryos. Gene Expr. Patterns, in press.

Shigenobu, S., Arita, K., Kitadate, Y., Noda, C., and Kobayashi, S. (2005). Isolation of germline cells from *Drosophila* embryos by flow cytometry. Develop. Growth Differ., in press.

Review article

Kobayashi, S., Sato, K., and Hayashi, Y. (2005). The role of mitochondrial rRNAs and Nanos protein in germline formation in *Drosophila* embryos. Zool. Sci. *22*, 943-954.