DIVISION OF DEVELOPMENTAL GENETICS

Professor:	*KOBAYASHI, Satoru*
Research Associates:	*MUKAI, Masanori*
	SHIGENOBU, Shuji
Technical Staff:	*NODA, Chiyo*
NIBB Research Fellow:	*ARITA, Kayo*
Postdoctoral Fellows:	*SATO, Kimihiro*
	HAYASHI, Yoshiki
Graduate Students:	*KITADATE, Yu*
	HAYASHI, Makoto
	YATSU, Jun
	MAEZAWA, Takanobu
	HASHIYAMA, Kazuya
Technical Assistants:	*UEDA, Yoshiko*
	TSUCHIYA, Naomi
	SATO, Kaori
Secretary:	*HONDA, Satoko*

Germ cells are the specialized cells that can transmit the genetic materials from one generation to the next in sexual reproduction. All of 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 development. In *Drosophila*, this cytoplasm is localized in the posterior pole region of eggs, and partitioned into the germline progenitors, or pole cells.

I. The role of maternal Nanos protein

In many metazoans, the germline forms early in development and is maintained until the differentiation of gametes in the adult gonads. Although genetic analyses have identified several mutations that eliminate pole cells, how pole cells are maintained during development is unclear.

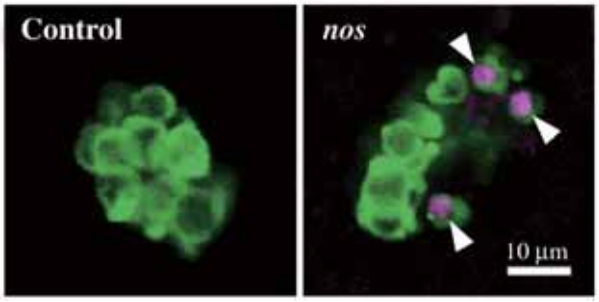

Figure 1. Nanos prevents apoptosis in pole cells. Confocal images of the pole cells in control (left) and *nos* (right) embryos at stage 13, stained with TUNEL labeling (magenta) and an antibody against Vas (green). Arrowheads show TUNEL-positive pole cells.

Several components of germ plasm have been identified. One of these components is maternal *nos* RNA, which is enriched in germ plasm during oogenesis and translated *in situ* to produce Nos protein after fertilization. While Nos is present transiently in the posterior half of embryos during the preblastoderm stage and is required for abdominal patterning, Nos in the germ plasm is inherited by pole cells at the blastoderm stage and is detectable in these cells throughout embryogenesis. In the absence of maternal Nos, pole cells undergo apoptosis during their migration to the embryonic gonads (Figure 1). Although Nos also plays important roles in repressing mitosis, somatic gene expression and somatic cell fate in pole cells, the primary role for Nos appears to be repressing apoptosis in the germline, because Nos is an evolutionarily conserved protein that is required for germline survival.

Nos is known to repress translation of specific RNAs that contain a discrete sequence called the Nos response element (NRE). In abdominal patterning, Nos represses the translation of maternal *hunchback* (*hb*) RNA. This repression requires NRE sequence. In pole cells, Nos represses translation of maternal *cyclin B* RNA that contains an NRE-like sequence within its 3'UTR. This repression results in the mitotic quiescence of pole cells during their migration to the gonads.

Apoptosis is mediated by caspases, a family of cysteine proteases that cleave diverse substrates to destroy cellular structure and integrity. Critical regulators of apoptosis function by antagonizing inhibitor of apoptosis protein (IAP) that directly blocks caspase action. In *Drosophila*, four proapoptotic genes, *reaper* (*rpr*), *head involution defective* (*hid*), *grim* and *sickle* (*skl*), encode members of a family of related proteins that bind to and inactivate the IAP. They are also referred to as RHG genes. Three of these RHG genes, *rpr*, *hid* and *grim*, are encompassed by a genomic region on the third chromosome, *H99*. Previously we have reported that deletion of *H99* region, *Df(3L)H99*, represses apoptosis of pole cells lacking Nos, consistent with a role for Nos in an apoptotic pathway that involves the RHG gene(s) from the *H99* region.

In this study, we demonstrate that maternal Nos represses apoptosis of pole cells by suppressing translation of *hid* RNA in an NRE-dependent manner. In the absence of Nos activity, translation of *hid* mRNA yields a protein product that induces apoptosis. In addition, we provide evidence that a maternally-provided protein kinase, Tao-1, is required to induce apoptosis in *nos* pole cells by promoting *skl* expression. Maternal *tao-1* RNA is enriched in the germ plasm and inherited by pole cells. Tao-1-dependent *skl* expression sensitizes pole cells to induction of apoptosis by *hid*. We propose that pole cells express the RHG genes *hid* and *skl*, and become competent to undergo apoptosis during normal development. However, maternal Nos represses *hid* translation to inhibit apoptosis of pole cells for their survival. These findings provide the first evidence that the germline is maintained through the regulated expression of RHG genes.

II. 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.

III. Molecular characterization of the embryonic gonads by gene expression profiling

Within the embryonic gonads, distinct cellular events associated with germline development occur, such as resumption of germline proliferation, selection of the germline-stem cell, gonad morphogenesis and cellular communication between the germline and somatic cells. Recent studies have also revealed that the male germline stem cell niche is already specified in the embryonic gonad. Despite the importance of the embryonic gonad in the germline development, only limited information is available regarding which genes are expressed in the embryonic gonad although transcriptome data of adult testes and ovaries has accumulated. Thus, we attempted to identify the genes expressed within the embryonic gonads by a direct and comprehensive approach. In *Drosophila*, transcriptome analysis of individual organs and cell types has been hampered by the smallness of their size. To overcome this problem, we have developed an efficient method to isolate embryonic gonads by flow cytometry.

First, we generated a cDNA library from gonads purified from *Drosophila* embryos by fluorescence-activated cell sorting (FACS). Using this library, we catalogued the genes expressed in the gonad by Expressed Sequence Tag (EST) analysis. A total of 17,218 high-quality ESTs representing 3,051 genes were obtained. This corresponds to 20% of the predicted genes in the genome. The embryonic-gonad transcriptome is unexpectedly distinct from that of adult gonads and includes an extremely high proportion of retrotransposon-derived transcripts. We verified 101 genes preferentially expressed in the embryonic gonads by whole-mount *in situ* hybridization (Figure 2). Within this subset, 39 and 58 genes were expressed predominantly in germline and somatic cells, respectively, while 4 genes were expressed in both cell lineages. The gonad-enriched genes encompassed a variety of predicted functions. However, genes implicated in SUMOylation and protein translation, including germline-specific ribosomal proteins, are preferentially expressed in the

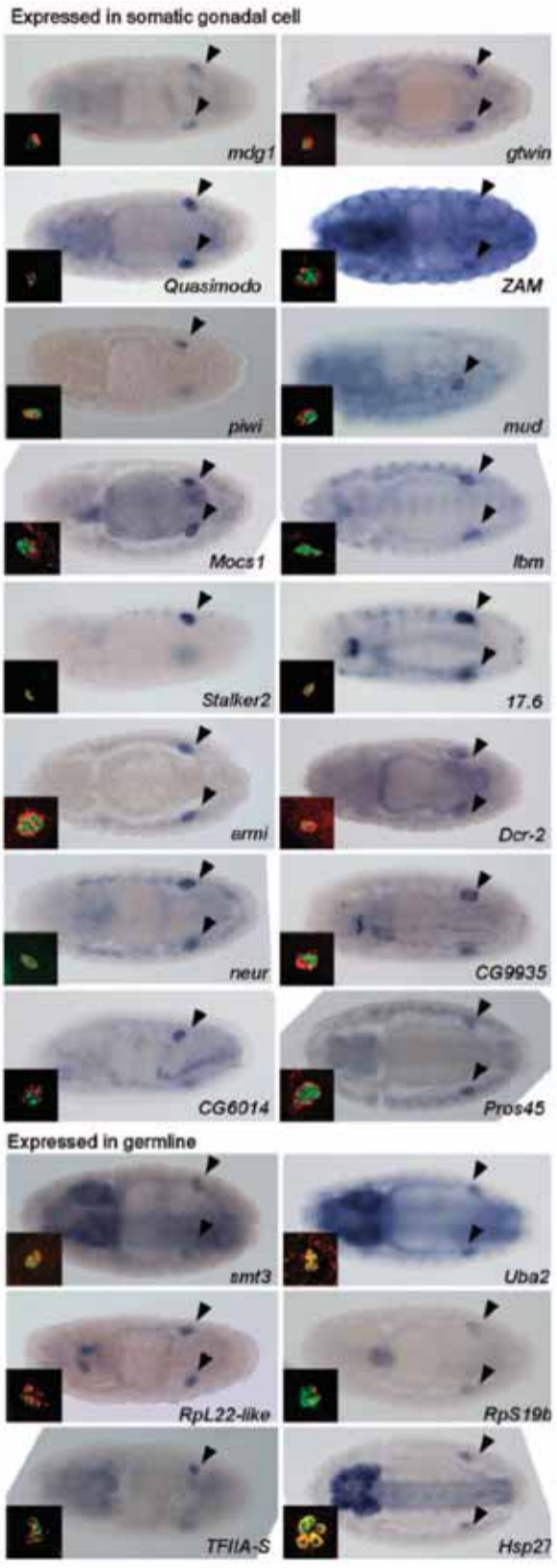

Figure 2. Spatial expression patterns of gonad-enriched transcripts. Embryos at stage 14-16 are shown with anterior to the left. Gonads are indicated by arrowheads. Inset in each panel provides the confocal microscopic image of the gonad double-stained with an antisense RNA probe for the indicated gene (red) and anti-Vasa (green), a germline marker.

germline, while the expression of various retrotransposons and RNA interference (RNAi)-related genes are more prominent in the gonadal soma. This transcriptome data is a resource for understanding the mechanism of various cellular events during germline development.

IV. Germline stem cell niche formation in male gonad

Stem cells possess the remarkable capacity to generate both daughter cells that retain a stem-cell identity and others that differentiate. Stem cells reside in dedicated cellular microenvironments, termed stem-cell niches, which dictate the stem cell identity, maintain the stem-cell population, and coordinate proper homeostatic production of differentiated cells. Recent studies have addressed molecular aspects of how niches define stem-cell identity and behavior through intercellular signaling. As these niches enable stem cells to maintain tissue homeostasis during development, growth, repair and aging, understanding the mechanisms that regulate formation of stem-cell niches during development is critical. These processes, however, remain largely uncharacterized.

The germline-stem-cell niche in *Drosophila* testes has emerged as a useful model system for studying stem cells. In the apical tip of the adult testes, the germline stem cells lie in intimate contact with somatic hub cells, known as the niche, which causes the stem cells to retain self-renewing potential. Germline stem cells divide to produce one daughter cell that remains associated with hub cells, while the other daughter cell detaches and initiates spermatogenesis.

Hub cells are derived from a subset of somatic gonadal cells (SGCs) that are located in the anterior region of male embryonic gonads. It has been reported that the antero-posterior cellular identities within the gonads is regulated by the homeotic genes, *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*). However, how the formation of hub progenitors is restricted in the anterior of embryonic gonads remains elusive. Previous observations also suggest that the germline may be required for proper hub formation in male gonads. In the absence of germline cells, an expansion of the hub population is observed.

We demonstrate that a receptor tyrosine kinase, Sevenless (Sev), provides a cue to ensure that the niche develops in the anterior region of the male embryonic gonads. Sev is expressed by somatic cells within the posterior region of the gonads, and is activated by a ligand, Bride of sevenless (Boss), which is expressed by the germline, to prevent ectopic niche differentiation in the posterior gonadal somatic cells. Our findings provide the first evidence that signal transduction from germline to soma is essential for the proper development of a stem-cell niche.

Publication List:

Original papers

Mukai, M., Kitadate, Y., Arita, K., Shigenobu, S., and Kobayashi, S. (2006). Expression of meiotic genes in the germline progenitors of Drosophila embryos. Gene Expr. Patterns *6*, 256-266.

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Shigenobu, S., Arita, K., Kitadate, Y., Noda, C., and Kobayashi, S. (2006). Isolation of germline cells from *Drosophila* embryos by flow cytometry. Develop. Growth Differ. *48*, 49-57.

Shigenobu, S., Kitadate, Y., Noda, C., and Kobayashi, S. (2006). Molecular characterization of embryonic gonads by gene expression profiling in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *103*, 13728-13733.

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