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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 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. Maternal Nanos protein is required in pole cells to repress their apoptosis

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.

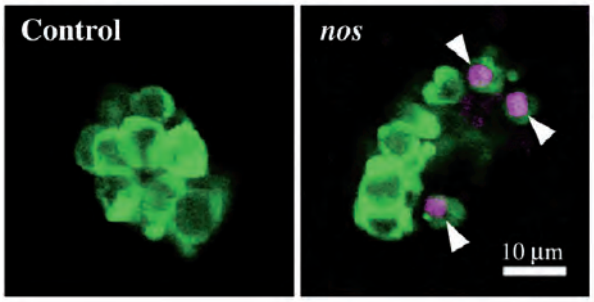

Figure 1. Nanos prevents apoptosis in pole cells. Confocal images of the pole cells in control (left) and *nos* (right) embryos at stage 13, stained with TUNEL labeling (magenta) and an antibody against Vas (green). Arrowheads show TUNEL-positive pole cells.

Several components of germ plasm have been identified. One of these components is maternal *nanos* (*nos)* RNA, which is enriched in germ plasm during oogenesis and translated *in situ* to produce Nos protein after fertilization. Nos 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.

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 the 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 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.

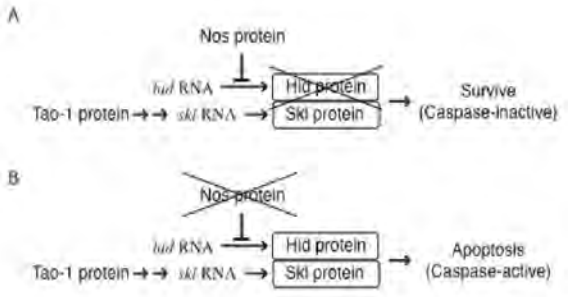

Figure 2. A model for the regulation of apoptosis in pole cells by the maternal factors, Nos and Tao-1. (A) In normal pole cells, maternal Tao-1 is inherited by pole cells and induces *skl* expression. Although *skl* alone does not induce apoptosis, it sensitizes pole cells to induction of apoptosis by *hid*. *Hid* mRNA is expressed in pole cells, but translation is repressed by maternal Nos. (B) Once pole cells lack Nos, hid mRNA is translated to produce its protein product that in turn acts together with *skl* to induce apoptosis.

We demonstrated 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 (Figure 2). These findings provide the first evidence that the germline is maintained through the regulated expression of RHG genes.

Ⅱ. Maternal Mamo protein is required in pole cells for their differentiation as the functional germ cells

Meiosis is a specialized cell division that produces haploid gametes from diploid progenitors. This is accomplished through two chromosomal segregation events without an intervening DNA replication. To accommodate the specialized processes, meiotic chromosomes undergo specific morphological changes (Figure 3). In *Drosophila* oocytes, upon entry into meiosis, a synaptonemal complex (SC) is formed between homologous chromosomes to stabilize their pairing, and the meiotic recombination occurs. Following disassembly of the SC, the meiotic chromosomes condense to form specialized prophase I chromosome structures, “karyosomes”, and these structures are maintained during later oogenesis. After ovulation, the oocyte initiates meiotic division and produces both a single female pronucleus and three polar-body nuclei which later fuse to form a rosette structure. These dynamic changes by the meiotic chromosomes are critical for meiosis. Genetic screens have identified many genes involved in the regulation of the meiotic chromosomes. The mechanism by which the germline acquires the potential to execute meiosis, however, remains elusive.

We demonstrated that a novel maternal factor, *mamo* (maternal gene required for meiosis), is autonomously required in pole cells to produce functional gametes. Mamo protein which contains both a BTB/POZ (Broad Complex, Tramtrack, Bric-a-brac/ Pox virus and Zinc finger) domain and C_2H_2 zinc finger motifs is enriched in PGCs during embryogenesis. The PGCs with reduced maternal Mamo activity are able to undergo oogenesis, but fail to execute meiosis properly (Figure 3). In the resulting oocytes, meiosis-specific chromosomal configurations are impaired (Figure 3). We additionally show that the decondensation of fertilized sperm nuclei is also affected in the eggs. We propose that maternal Mamo activates downstream genes to promote specialized morphological changes within both the female meiotic chromosomes and the sperm nucleus, which are critical in zygote formation.

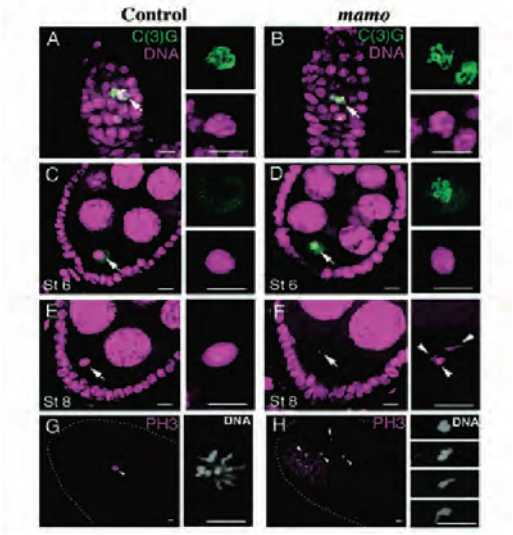

Figure 3. Maternal *mamo* mutation affects the structure of the SC, karyosome, and polar body. (A-F) Germarium regions (A and B), Stage-6 (C and D) and stage-8 (E and F) egg chambers were stained for C(3)G (green), a marker of the SC, and DNA (propidium iodide, magenta). (Insets) higher-magnification images of the oocyte nuclei are shown. (A and B) In the germarium region, C(3)G protein localized to the DNA in both the control and the *mamo* oocytes (arrows). (C and D) C(3)G was not associated with the DNA, and was diffusely distributed throughout the nuclear matrix (arrow) in a control oocyte at stage 6 (C). C(3)G failed to dissociate from the DNA (arrow) in a *mamo* oocyte (D). (E and F) The karyosome was compact and spherical in a control oocyte at stage 8 (arrows) (E), while the karyosome was fragmented (arrow) in a *mamo* oocyte at stage 8 (F). (G, H) Chromosomes were stained with an anti-PH3 antibody and TOTO-3 in control (G) and *mamo* eggs (H) at 0-1 hr AEL. (G) In a control egg, the rosette structure derived from polar bodies was observed (arrow). (H) In a *mamo* egg, rosette structure was indiscernible. The chromosomes were scattered, and their number was significantly reduced. (Insets) Higher-magnification images of the chromosomes shown in (G) and (H). Dotted lines outline the eggs. Scale bars: 10 μm.

Ⅲ. Signaling from pole cell to the gonadal soma is required for proper formation of the germline-stem-cell niche

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 collectively 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*). How the formation of hub progenitors is restricted in the anterior of embryonic gonads, however, remains elusive. We demonstrate that a receptor tyrosine kinase, Sevenless (Sev), provides a cue to ensure that the niche

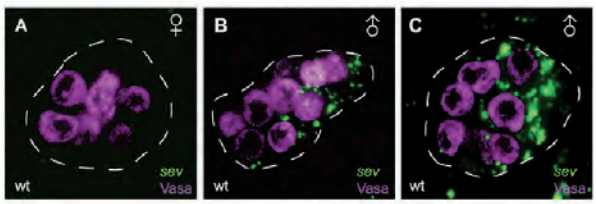

Figure 4. Expression of *sev* within the embryonic gonads
The gonads in a female embryo at stage 16 (A) and in male embryos at stage 13 (B) and 16 (C). Embryos were double-stained for *sev* mRNA (green) and a marker for the germline, Vasa (magenta). In all panels, anterior is to the left, and the embryonic gonads are outlined by white lines.

develops in the anterior region of the male embryonic gonads. Sev is expressed by somatic cells within the posterior region of the gonads (Figure 4), 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.

Ⅳ. Studies on short ORF-containing transcripts in *Drosophila* (Kageyama group)

Transcriptome analyses of eukaryotes, including mice and humans, have identified poly(A)-containing transcripts that only contains short ORFs (sORFs; less than 100 aa). These sORF transcripts are believed to most likely function as non-coding RNAs (ncRNAs), but their translational capacities and biological activities have not been characterized in detail.

To elucidate the biological roles of sORF genes, we use *Drosophila* as an excellent model system. By computational filtration and expression analysis, we have identified 33 putative poly(A)-containing ncRNAs, referred as to MREs (mRNA-like ncRNAs in embryos). In this year, we have been focusing on the biological roles of two MRE genes, MRE32 and MRE29. MRE32 is specifically expressed in the central nervous system and detailed transcript mapping strongly suggests that it really functions as ncRNA. Mutational analysis revealed that the *MRE32* gene is required for the eclosion behavior of the flies. Eclosion timing is sexually dimorphic in *Drosophila melanogaster*, in which females eclosed one day earlier than males. In *MRE32* mutant flies, female eclosion was specifically delayed, resulting in similar eclosion profiles in both sexes. In addition, microarray analysis of female and male adult heads demonstrated that a lack of *MRE32* severely altered expression of many genes in females. Thus, MRE32 is one of the crucial regulators of the Drosophila nervous system that acts on developmental timing. Another MRE, MRE29, is in fact transcribed into a polycistronic mRNA that contains evolutionarily conserved four ORFs that encode tiny peptides (11 and 32 aa). *MRE29* is expressed in epithelial tissues during embryogenesis and a lack of the *MRE29* gene eliminated apical cuticular structures, including the epidermal denticles and tracheal taenidia (Figure 5). Considering these phenotypes, we renamed the gene as *polished rice* (*pri*). Cytological analysis demonstrate that *pri* is essential for the formation of specific F-actin bundles that prefigure the formation of the denticles and taenidium (Figure 5), indicating that *pri* plays essential roles in epithelial morphogenesis by regulating F-actin organization.

These results demonstrate that sORF genes play important roles in *Drosophila* and further analysis on other MRE members should elucidate unexplored functions of the *Drosophila* genome.

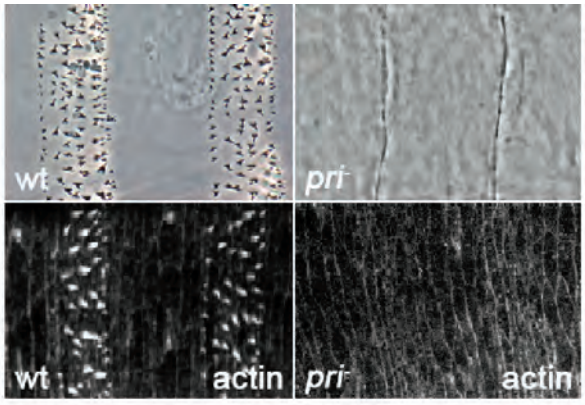

Figure 5. Epidermal phenotype of *polished rice*. In contrast to wild-type embryos (left column), pri mutant embryos exhibit complete loss of denticles (right, upper panel) and actin bundle formation beneath denticles (right, lower panel).

Publication List

〔Original papers〕

- Kitadate, Y., Shigenobu, S., Arita, K., and Kobayashi, S. (2007). Boss/Sev signaling from germline to soma restricts germline-stem-cell-niche formation in the anterior region of *Drosophila* male gonad. Dev. Cell *13*, 151-159.
- Kondo, T., Hashimoto, Y., Kato, K., Inagaki, S., Hayashi, S., and Kageyama, Y. (2007). Small peptide regulators of actin-based cell morphogenesis encoded by a polycistronic mRNA. Nature Cell Biol. *9*, 660-665.
- Mukai, M., Hayashi, Y., Kitadate, Y., Shigenobu, S., Arita, K., and Kobayashi, S. (2007). MAMO, a maternal BTB/POZ-Zn-finger protein enriched in germline progenitors is required for the production of functional eggs in *Drosophila*. Mech. Dev. *124*, 570-583.
- Nakamura, Y., Kagesawa, T., Nishikawa, M., Hayashi, Y., Kobayashi, S., Niimi, T., and Matsuno, K. (2007). Soma-dependent modulations contribute to divergence of rhomboid expression during evolution of *Drosophila* eggshell morphology. Development *134*, 1529-1537.
- Sato, K., Hayashi, Y., Ninomiya, Y., Shigenobu, S., Arita, K., Mukai, M., and Kobayashi, S. (2007). Maternal Nanos represses *hid/skl*-dependent apoptosis to maintain the germ line in Drosophila embryos. Proc. Natl. Acad. Sci. USA *104*, 7455-7460.