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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 germline stem cells to form eggs and sperm when the organisms are physically matured.

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

Several components of germ plasm have been identified in *Drosophila*. 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 primordial germ cells or 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. 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.

We have demonstrate that maternal Nos represses apoptosis of pole cells by suppressing translation of *hid* RNA. In the absence of Nos activity, translation of *hid* mRNA yields a protein product that induces apoptosis. In addition, 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 *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.

We recently found that *hid* expression requires *eiger* (*egr*), a tumor necrosis factor (TNF) homolog, that is induced in pole cells by *decapetaplegic* (*dpp*). In addition, *p53* and *loki* (*lok*), a damage-activated kinase known to be required for phosphorylation of p53, are both required for hid expression in pole cells. Since maternal lok mRNA is enriched in germ plasm and then partitioned into pole cells, we speculated that ubiquitously distributed p53 may be activated in pole cells by maternal Lok. Taken together, we propose that *hid* expression is activated in a pole cell-specific manner by *loki/p53* and *dpp/egr* during normal embryogenesis (Figure 1).

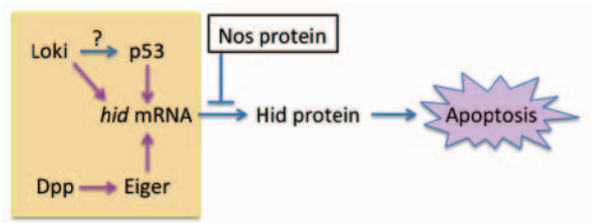

Figure 1. A model for the regulation of *hid* mRNA expression and apoptosis in pole cells.

Ⅱ. Expression of genes involved in sumoylation in pole cells

To identify the genes essential for germline development, we have performed EST and microarray analyses using pole cells and embryonic gonads. During the course of this analysis, we noticed that five genes in the SUMO (small ubiquitin-related modifier) conjugation pathway are highly expressed in pole cells and embryonic gonads (Hashiyama and Kobayashi, Gene Expression Patterns, *9*, 50-53, 2009).

Sumoylation regulates a wide range of cellular processes including transcription, genomic replication, nucleocytoplasmic signaling, and chromatin dynamics. SUMO proteins are covalently attached to lysine residues in the substrate protein by a series of enzymatic reactions similar to the ubiquitination system. Briefly, SUMO protein is processed by a SUMO-specific protease, and a SUMO-activating enzyme activates and transfers the SUMO protein to the SUMO-conjugating enzyme. SUMO is then covalently attached to the substrate protein by a SUMO protein ligase. We analyzed the spatiotemporal expression

patterns of the genes encoding the SUMO protein (*smt3*), the SUMO-specific protease (*Ulp1*), the SUMO-activating enzymes (*Uba2* and *Aos1*), and the SUMO-conjugating enzyme Ubc9 (*lesswright, lwr*) in *Drosophila* embryos and adult gonads (Figure 2).

Transcripts from all five genes are detected throughout the early embryo by whole mount in situ hybridization, while they are predominantly expressed in pole cells in late stage embryos. These genes are also expressed in the germline during oogenesis and spermatogenesis. We also found that SUMO protein is enriched in the nuclei of pole cells and gametogenic cells. Given that a large fraction of SUMO substrates are nuclear proteins, this data suggests that sumoylation is highly active in the germline. Our data provide a basis for understanding how sumoylation regulates germline development.

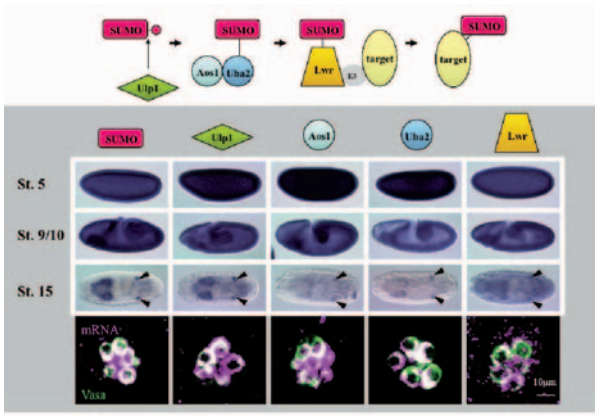

Figure 2. Expression of sumoylation genes in embryos.
In situ hybridization of embryos with antisense probes for *smt3* (sumo), *Ulp1*, *Uba2*, *Aos1* and *lwr*. Embryos at stage 5, stage 9/10 and stage 15 are shown. Anterior is to the left. Arrows indicate gonads.

Ⅲ. Mechanism leading to sexual dimorphism of pole cells

Germ cells must develop along distinct male or female paths to produce eggs or sperm. It has been reported that germline sexual identity is regulated by a masculinizing signal from the somatic gonadal cells (SGCs) to pole cells within the embryonic gonads. However, we found that reduction of sumoylation causes apoptosis of the migrating pole cells in a female-specific manner, suggesting that sexual identity has already been established in the pole cells prior to gonad formation. We also found that *Sex lethal* (*Sxl*) is expressed in the migrating pole cells in a female-specific manner, and its function is required for the feminization of pole cells. Ectopic expression of *Sxl* in XY (male) pole cells is able to direct the developmental fate of these cells to become functional eggs. Our results show that the germline-autonomous mechanism, along with the non-autonomous mechanism, leads to sexual dimorphism in the germline (Figure 3).

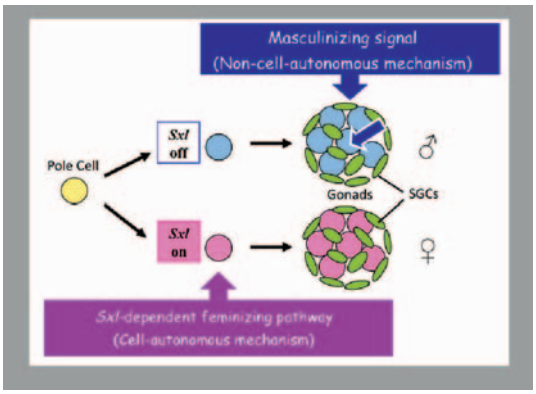

Figure 3. A model for the regulation of sexual dimorphism in pole cells.

Ⅳ. Mechanism regulating the formation of the niche cells in male embryonic gonads

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

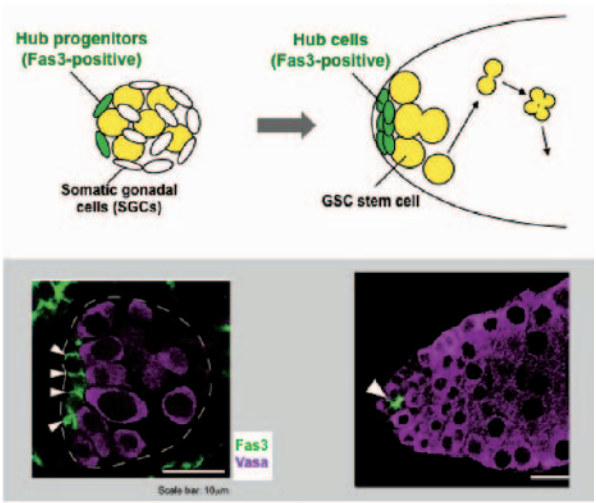

Figure 4. Hub and hub progenitors are marked with a molecular marker, Fasciclin3 (Fas3). Fas3 positive cells (green) are located in the anterior tips of both adult and embryonic gonads. Magenta shows germline cells.

Hub cells are derived from a subset of somatic gonadal cells (SGCs) that are located in the anterior region of male embryonic gonads (Figure 4). How the formation of hub progenitors is restricted in the anterior of embryonic gonads, however, remains elusive. We have demonstrated that a receptor tyrosine kinase, Sevenless (Sev), provides a cue to ensure that the hub cells develops in the anterior region of the male embryonic gonads. Sev is expressed by SGCs within the posterior region of the gonads, and is activated by a ligand, Bride of sevenless (Boss), which is expressed by pole cells, to prevent ectopic hub differentiation in the posterior SGCs.

Recently, we found that Egfr, like Sev, is activated by a ligand from pole cells to prevent hub differentiation in the posterior SGCs. We also showed that hub formation requires Notch, which is activated in almost all of the SGCs within the male embryonic gonads. Thus, we propose that almost all of the male SGCs become competent to form hub by the function of Notch, but niche formation is repressed in the posterior SGCs by Egfr and Sev, thereby restricting niche in the anterior region.

Ⅴ. The role of heparan sulfate proteoglycan in the germline-stem-cell niche

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 niches. These niches dictate a stem-cell identity, maintain the stem cell population, and coordinate proper homeostatic production of differentiated cells. Recent studies have shown that several signaling pathways, such as TGF-beta and JAK/ STAT are essential for maintenance of the *Drosophila* GSCs. GSCs are surrounded by somatic cells (niche cells) which form the physical area of the niche and are responsible for signaling molecule secretion. Despite the importance of these molecules for GSC maintenance, it is not yet well understood how these secreted molecules can precisely define the region of GSC niche.

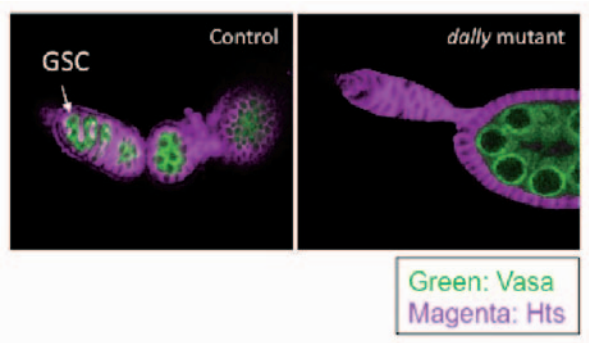

Figure 5. HSPG is essential for GSC maintenance in female gonads. In HSPG mutant (*dally*), GSCs are missing from the anterior tip of the ovary. Green and magenta show germline cells and somatic cells, respectively.

Heparan Sulfate Proteoglycans (HSPGs) are a glycoprotein which are expressed on the cell surface and/ or in the extracellular matrix. Recent *in vivo* studies have shown that HSPGs play critical roles in regulating signaling during development by controlling extracellular ligand distribution. For example, one of the *Drosophila* glypicans, *dally*, can control the distribution of a TGF-beta ligand, Dpp, and establish Dpp gradient during wing development.

We showed that HSPGs are essential for GSC maintenance in both male and female gonads. In the HSPG mutant gonads, GSCs could not maintain their number and lost the characteristics of undifferentiated germ cells (Figure 5). In both male and female, HSPG genes are expressed in the GSC niche cells. We present the model that HSPGs have a role in defining the GSC niche by controling ligand stability or distribution in[the niche region.

Publication List

〔Original paper〕

- Yatsu, J., Hayashi, M., Mukai, M., Arita, K., Shigenobu, S., and Kobayashi, S. (2008). Identification of maternal RNAs encoding transcription factors required for germline-specific gene expression in *Drosophila* embryos. Int. J. Dev. Biol. *52*, 913-923.