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Germ cells are specialized cells that can transmit 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 (GSC) to form eggs and sperm when the organisms are physically matured. Our laboratory aims to find the molecular mechanisms regulating germline segregation, germline sex determination and GSC niche formation and function in *Drosophila*.

I. Genome-wide search for RNAs of which translation is regulated by Nanos in the germline of *Drosophila* embryos

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.

Nos acts as a translational regulator for specific RNAs in the pole cells. Maternal Nos represses apoptosis and mitosis of pole cells by suppressing translation of *cyclin-B* and *head involution defective* RNA, respectively. Moreover, Nos is required for the repression of somatic cell fate in the pole cells and for the germline development within the gonads, presumably via regulating unidentified RNAs. Thus, we started a genome-wide identification of RNAs of which translation is regulated by Nos in pole cells. Nos is known to function together with the Pumilio (Pum) protein, which directly binds to distinct sequence in 3'-UTR of the target mRNAs. Recently, Gerber et al. have reported genome-wide identification of 165 Pum-binding RNAs. Based on this data, we started a systematic screen to identify target mRNAs for Nos/Pum-dependent translational regulation in pole cells. We expressed hybrid mRNAs containing GFP-coding region and 3'-UTR sequence from the Pum-binding RNAs, and then examined GFP expression in the pole cells with or without maternal Nos activity. Among twenty hybrid mRNAs, six were translationally repressed by Nos. In addition, we found that translation of two mRNAs were up-regulated by Nos. We are now examining the roles of these mRNAs in pole cell development.

II. Mechanism regulating sex determination 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 to pole cells within the embryonic gonads. However, we have 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.

Here we show that Sex lethal (Sxl) acts autonomously in the germline to induce female development in Drosophila. Sxl is transiently expressed in the germline progenitors, or pole cells, during their migration to the gonads. Its expression is detected in a female-specific manner and is necessary for feminization of pole cells before they form the gonads. Furthermore, ectopic expression of Sxl in male (XY) pole cells is sufficient to induce female fate in these cells, and the resulting pole cells are able to produce functional eggs within female (XX) soma.

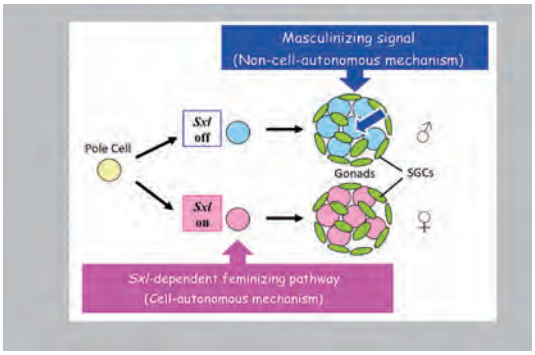

Figure 1. A model for the regulation of sexual dimorphism in pole cells.

The germline sexual identity is regulated both by sex of the surrounding soma and by a cell-autonomous cue. Our findings provide strong evidence that Sxl has a pivotal role in the germline-autonomous mechanism regulating sex determination. XX pole cells initiate female sexual identity based on their Sxl expression, while, lacking Sxl expression in XY pole cells, male sexual fate occurs primarily by a signal from gonadal soma (Figure 1). One remarkable

example of germline-autonomous regulation of sexual dimorphism has been reported in a primitive animal, cnidarian *Hydra*. It has long been known that sex of the germline is not influenced by the surrounding soma, and the germline, rather than soma, determines the phenotypic sex of the polyp. Thus, we speculate that germline-autonomous regulation of sex is a primitive trait conserved throughout the evolution of animals, and somatic control may have evolved with the emergence of mesodermal tissues, including gonadal soma.

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

The 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.

Hub cells are derived from a subset of somatic gonadal cells (SGCs) that are located in the anterior region of male embryonic gonads. However, it remains unclear how the proper niche size and location are regulated within the developing gonads. We have demonstrated that a receptor tyrosine kinase, Sevenless (Sev), represses hub development in the anterior region of 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.

We further found that Notch signaling induces hub differentiation. Notch is activated in almost all of the SGCs within male embryonic gonads, suggesting that the posterior SGCs, as well as the anterior SGCs, have the capacity to contribute to hub differentiation. Since hub differentiation is restricted in the anterior SGCs, the posterior SGCs should be repressed to become hub cells. Although Sev acts as a repressor for hub differentiation in the posterior SGCs, expression of a constitutive-active form of Sev is unable to inhibit hub differentiation observed in the anterior SGCs, suggesting that Sev does not have a sufficient ability to repress hub differentiation. Thus we speculate that another RTK signaling pathway has a key role to restrict hub differentiation in the anterior SGCs.

We showed that epidermal growth factor receptor (Egfr) is activated in the posterior SGCs to repress hub differentiation. In the absence of *Egfr* activity, ectopic niche differentiation is evident in the posterior SGCs. Moreover, hub differentiation which is normally observed in the anterior SGCs was repressed by expressing a constitutively active form of *Egfr* throughout SGCs. These observations show that Egfr is both required and sufficient to repress hub differentiation.

Egfr is activated in the posterior SGCs by Spitz ligand emanating from pole cells, while a ligand for Notch, Serrate, is expressed in SGCs (Figure 2). This implies that varying the number of pole cells alters the niche size. Indeed, a decrease in the number of pole cells causes ectopic hub differentiation, which consequently increases their chance to recruit pole cells as GSCs. When ectopic hub differentiation is repressed, the decreased number of pole cells fail to become GSCs. Thus we propose that SGCs sense PGC number by the signaling from PGCs to SGCs to modulate niche size, and this serves as a mechanism securing GSCs (Figure 3).

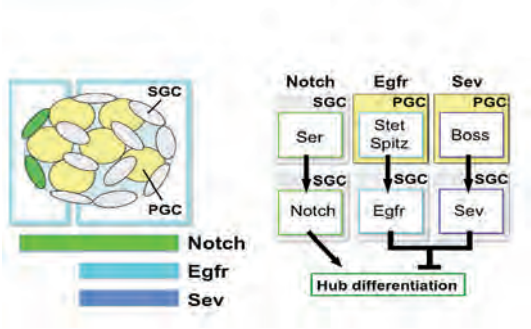

Figure 2. Hub differentiation is controlled by negative regulators, Sev and Egfr and a positive regulator, Notch.

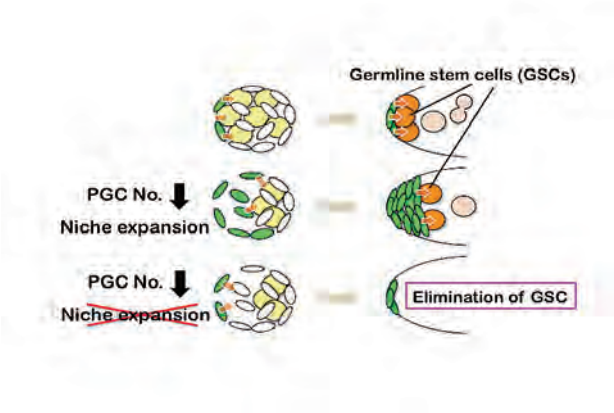

Figure 3. Mechanism securing the germline stem cells. Decrease in PGC number causes niche expansion, thereby recruiting a small number of PGCs as GSCs.

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

Stem cells posses the remarkable capacity to generate daughter cells that retain a stem-cell identity and others that differentiate. Stem cells reside in dedicated cellular microenvironments termed stem-cell niches. These niches dictate stem-cell identity, maintain the stem cell population, and coordinate proper homeostatic production of differentiated cells. *Drosophila* GSCs are one of the most characterized among animal stem cells. Recent studies show that several signaling pathways, such as TGF-beta and JAK/STAT are essential for maintenance of the GSC niche. GSCs are surrounded by somatic gonadal cells, named as niche cells, which form the physical area of the niche and are

responsible for signaling molecule secretion. Despite the importance of these molecules, it is not well understood how these secreted molecules can precisely define the region of the GSC niche.

Heparan Sulfate Proteoglycans (HSPGs) are a group of glycoproteins 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 a variety of mechanisms, including controlling extracellular ligand distribution. For example, one of the *Drosophila* glypicans, *dally* can control distribution of the TGF-beta ligand, Dpp, and establish a Dpp morphogen gradient during wing development.

We recently identified the *Drosophila* glypicans, *dally* and *dally-like* as important components of the GSC niche in both sexes. *dally* and *dally-like* were strongly expressed in female and male GSC niche cells, respectively. Mutant animals for these glypicans showed significant reduction of GSC number as a result of failure of proper signal activation. Furthermore, ectopic expression of *dally* in female gonads caused an increase in GSC number. Based on these observations, we conclude that glypicans define GSC niche region by regulating signaling pathways involved in GSC maintenance. We present the model that glypicans have a role in defining the GSC niche by controlling ligand stability or distribution in the niche region.

Figure 4. *dally* is an essential component of the GSC niche. (A-C) Anterior most region of ovariole (Germarium) from control (A), *dally* mutant (B) and *dally*-ectopically-expressed animal (C). In control female, two to three GSCs (arrows) exist at the anterior tip of the germarium (A). Once *dally* function is lost, GSC number is decreased, and consequently germarium lacking GSCs are frequently observed (B, bracket). Conversely, germarium in which *dally* is ectopically expressed possess more than ten GSCs (C, bracket). Green signal shows germline marker, Vasa protein, and magenta signal shows membrane-skeletal protein, Hts. GSCs are identified as spherical shaped Vasa positive cells with dot shaped Hts signals.

V. Transcriptional regulation in Malaria parasites (S. Shigenobu)

Gene expression in Plasmodium parasites undergoes significant changes in each developmental stage, but the transcription factors (TFs) regulating these changes have not been identified. We identified a Plasmodium TF (AP2-O) that activates gene expression in ookinetes, the mosquito-invasive form, and has a DNA-binding domain structurally related to that of a plant TF, Apetala2 (AP2). The Plasmodium TF activates a set of genes, including all genes reported to be required for midgut invasion, by binding to specific six-base sequences on the proximal promoter.

Publication List

〔Original papers〕

- Hashiyama, K., and Kobayashi, S. (2009). Expression of genes involved in sumoylation in the *Drosophila* germline. Gene Expression Patterns *9*, 50-53.
- Hayashi, Y., Kobayashi, S., and Nakato, H. (2009). *Drosophila* glypicans regulate the germline stem cell niche. J. Cell Biol. *187*, 473-480.
- Maezawa, T., Arita, K., Shigenobu, S., and Kobayashi, S._(2009). Expression of the apoptosis inducer gene *head involution defective* in primordial germ cells of the *Drosophila* embryo requires *eiger, p53* and *loki* function. Develop. Growth Differ. *51*, 453-461.
- Yuda, M., Iwanaga, S., Shigenobu, S., Mair, G., Janse, C., Waters, A., Kato, T., and Kaneko I. (2009). Identification of a transcription factor in the mosquito-invasive stage of malaria parasites. Mol. Microbiol. *71*, 1402-1414.