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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 function in *Drosophila*.

I. Role of maternal Ovo protein in the germline of *Drosophila* embryos

It has been proposed that germline-specific gene expression is initiated by the function of maternal factors that are enriched in the germ plasm. However, such factors have remained elusive. Here, we describe a genome-wide survey of maternal transcripts that encode for transcription factors and are enriched in the germ plasm. We isolated pole cells (primordial germ cells; PGCs) from blastodermal embryos by fluorescence-activated cell sorting (FACS) and then used these isolated cells in a microarray analysis. Among the 835 genes in the Gene Ontology (GO) category "transcription regulator activity" listed in FlyBase, 68 were found to be predominantly expressed in PGCs as compared to whole embryos. As the early PGCs are known to be transcriptionally quiescent, the listed transcripts are predicted to be maternal in origin. Our *in situ* hybridization analysis revealed that 27 of the 68 transcripts were enriched in the germ plasm. Among the 27 transcripts, six were found to be required for germline-specific gene expression of *vasa* and/or *nanos* by knockdown experiments using RNA interference (RNAi).

Among the 6 transcripts, we focused on *ovo*. The *ovo* gene encodes for a DNA-binding, C2H2 Zn-finger protein that is involved in oogenesis and in epidermal development. The *ovo* gene produces at least three alternate isoforms. Ovo-A and Ovo-B function as a negative and a positive transcriptional regulator in the germline, respectively. Ovo-Svb is expressed in the epidermal cells and is required for their differentiation. We found that Ovo-B is the major isoform expressed in PGCs during embryogenesis. To understand its function, we over-expressed the Ovo-A repressor only in PGCs, and examined their developmental fate. Our data shows that the reduction in maternal Ovo-B activity results in a decrease in the number of primordial germ cells during post-embryonic stages, which thereby causes sterility in both female and male adults. Thus, maternal Ovo-B has an essential role in germline development in both sexes.

II. Mechanism regulating sex determination of PGCs

"Sex reversal" leads to infertility, because the soma and germ cells become incompatible; male gonads are not able to deliver eggs, and vice versa. Various genetic conditions result in sex change in the soma, but in these cases germ cells retain their original gender, and how sex is determined in germ cells has remained unclear. It is widely accepted in mammals and *Drosophila* that male sexual development is imposed in PGCs by the sex of the gonadal soma, and that PGCs assume a female fate in the absence of a masculinizing environment. How PGCs initiate female development, however, is a long-standing question in reproductive and developmental biology.

In the soma, sex determination is controlled by the *Sex lethal (Sxl)* gene, which is first expressed at the blastodermal stage. *Sxl* encodes an RNA binding protein involved in alternative splicing and translation. In the soma of female (XX) embryos, it functions through *transformer* (*tra*) and *transformer-2* (*tra-2*), which in turn regulate alternative splicing of the *doublesex* (*Dsx*) gene to produce a female-specific form of Dsx. In male (XY) embryos, this pathway is turned off and a male-specific form of Dsx is produced by default. These Dsx proteins determine the sexual identity of somatic tissues. Previous reports, however, suggested that *Sxl* does not induce female sexual development in the germline, as it does in the soma. Although *Sxl* is autonomously required for female sexual development, constitutive mutations in *Sxl* (Sxl^{M}) that cause XY animals to undergo sexual transformation from male to female does not necessarily interfere with male germline development. Moreover, *tra*, *tra-2*, and *dsx* are not required for female germline development. Finally, female-specific *Sxl* expression has been detected later in gametogenesis, but not in early germline development.

Contrary to the previous observations, we found that *Sxl* was expressed in XX, but not XY PGCs, during their migration to the gonads. Furthermore, we found that the Sxl^{M1} mutation does not result in *Sxl* expression in XY PGCs,

as early as in XX PGCs. To determine whether *Sxl* induces female development in XY PGCs, we then induced *Sxl* expression in XY PGCs using *nanos-Gal4* and *UAS-Sxl*. We transplanted three types of XY PGCs (Figure 1), each characterized by a different duration of *Sxl* expression: (i) XY PGCs in which *Sxl* was expressed from stage 9 until stage 16/17 using maternal *nanos-Gal4* (XY-mSxl), (ii) XY PGCs in which *Sxl* was expressed from stage 15/16 onward using zygotic *nanos-Gal4* (XY-zSxl), and (iii) XY PGCs in which *Sxl* was expressed from stage 9 onward using both maternal and zygotic *nanos-Gal4* (XY-mzSxl). We found that XY-mzSxl and XY-mSxl PGCs entered the oogenic pathway and produced mature oocytes in XX females. These oocytes contributed to progeny production. In contrast, XY-zSxl PGCs did not enter the oogenic pathway. These observations demonstrate that *Sxl* expression in XY PGCs during embryogenesis induces functional egg differentiation in the female soma.

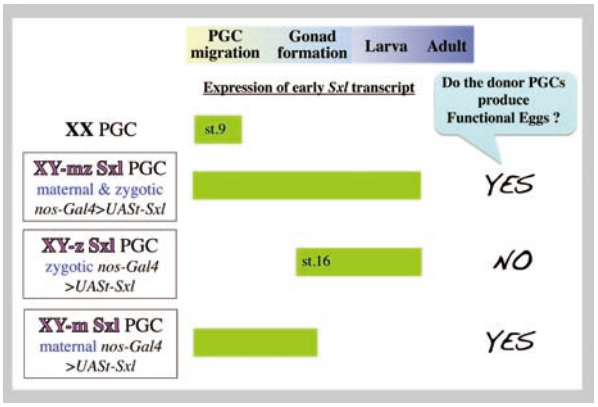

Figure 1. Expression of *Sxl* is sufficient to induce female fate in XY PGCs.

In contrast, *Sxl*-specific double-stranded RNA (*UAS-Sxl*^{RNAi}) under the control of maternal *nanos-Gal4* was then used to reduce *Sxl* activity in XX PGCs during embryogenesis. Introducing *UAS-Sxl*^{RNAi} resulted in tumorous and agametic phenotypes in female adults, indicating that the XX germline lost female characteristics.

Our findings provide powerful evidence for *Sxl* as a master gene that directs a female germline fate. XX PGCs initiate female sexual identity based on their *Sxl* expression, while, lacking *Sxl* expression in XY PGCs, male sexual fate occurs primarily by a signal from gonadal soma (Figure 2). 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.

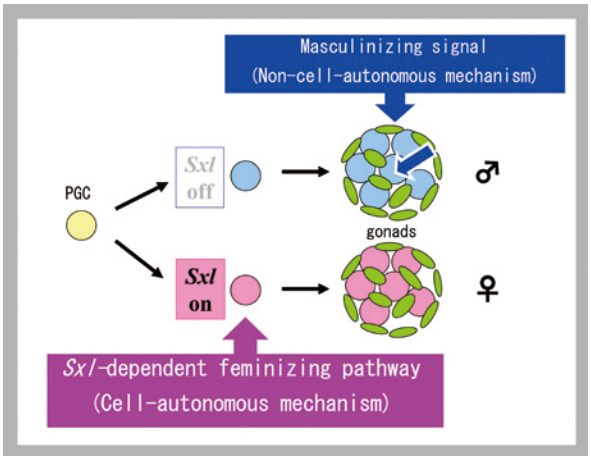

Figure 2. A model for the regulation of sexual dimorphism in pole cells.

III. The role of heparan sulfate proteoglycans 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. The germline-stem-cell (GSC) niche in *Drosophila* gonads is a useful model system for studying the stem-cell niche, because the cellular components of this niche have been characterized and the signaling pathways, such as TGF-beta and JAK/STAT, which are essential for GSC maintenance, are known. Ligands for these signaling pathways (niche signals) are secreted from the niche cells, and are received by GSCs to activate the pathway responsible for GSC maintenance. Thus, GSC niche is defined as the specialized region retaining a sufficient amount of niche signals for GSC maintenance. However, it is not well understood how the distribution of the niche signals is precisely controlled in 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 studies have revealed that HSPGs play critical roles in regulating signaling pathways during development by controlling extracellular ligand distribution. For example, one of the *Drosophila* glypicans, *dally* controls distribution of TGF-beta ligand, or Dpp, and thereby establishing a Dpp gradient during wing development.

We recently identified *Drosophila* glypicans, *dally* and *dally-like* as important components of GSC niche in both sexes. Mutations for these glypicans caused a significant reduction in GSC number, due to the failure of proper activation of the signaling pathway for GSC maintenance. Conversely, ectopic expression of *dally* in female gonads caused an increase in GSC number. These results strongly suggest that these glypicans define GSC niche by regulating distribution of niche signals.

To address this possibility, we have been trying hard to visualize niche signals in the GSC niche. By modifying

protocols for antibody staining and generating new antibodies, we succeeded in visualization of niche signal distribution. We found that Dpp distribution was significantly expanded when *dally* was ectopically expressed in female gonads, while Dpp-producing cells were unaffected (Figure 3). The above results support our model that glypicans define the GSC niche by regulating extracellular distribution of niche signals.

We also succeeded in detecting spatial distribution of JAK/STAT ligand, Upd (Figure 3). Upd is known to act as a morphogen for proper formation of egg chambers, as well as a niche signal in male GSC maintenance. We found that *dally* was able to affect Upd distribution within the egg chambers. This is the first evidence supporting the role of glypican in regulating extracellular distribution of Upd. Experiments testing whether glypicans regulate Upd distribution in male GSC niche are now ongoing.

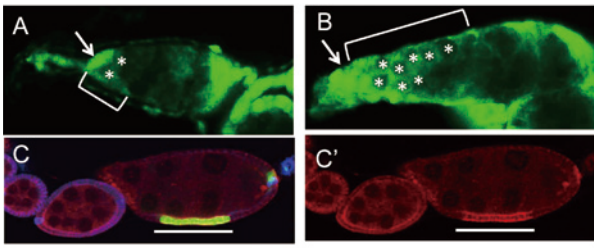

Figure 3. Glypican regulates spatial distribution of niche signals.
(A,B) Dpp distribution in distal tip region (germarium) of normal ovary (A) and of ovary expressing *dally* in the somatic cells throughout germarium (B). Green signal indicates Dpp protein (Blackets). Dpp distribution is expanded in *dally*-expressing ovary, compared to that observed in normal ovary. Arrows show niche cells, which are the source of Dpp. Asterisks indicate GSCs. (C, C') Distribution of Upd in egg chamber. Upd (red signal) is ectopically accumulated in *dally*-expressing clone (green, Bar).

Publication List

〔Original papers〕

- Hashiyama, K., Hayashi, Y., and Kobayashi, S. (2011). *Drosophila Sex lethal* gene initiates female development in germline progenitors. Science *333*, 885-888.
- Mukai, M., Kato, K., Hira, S., Nakamura, K., Kita, H., and Kobayashi, S. (2011). Innexin2 gap junctions in somatic support cells are required for cyst formation and for egg chamber formation in *Drosophila*. Mech. Dev. *128*, 510-523.