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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. We have done a genome-wide survey of maternal transcripts that are enriched in the germ plasm and encode transcription factors for germline-specific gene expression of *vasa* and/or *nanos*. We finally identified 6 transcripts required for germline-specific gene expression 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. Thus, maternal Ovo-B has an essential role in germline development in both sexes. Experiments for identifying the downstream genes regulated by Ovo-B in germline are now on-going.

II. Mechanism regulating sex determination of PGCs

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.

We have reported that *Sex lethal* (*Sxl*) was expressed in XX female, but not XY male PGCs, during their migration to the gonads. To determine whether *Sxl* induces female development in XY PGCs, we induced *Sxl* expression in XY PGCs using *nanos-Gal4* and *UAS-Sxl*, and transplanted these PGCs into XX females. We found that these XY PGCs entered the oogenic pathway and produced mature oocytes in XX females. These oocytes contributed to progeny production. In contrast, XY 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.

Our findings provide powerful evidence for *Sxl* as a master gene that directs a female germline fate. Experiments for identifying the downstream genes regulated by *Sxl* and the upstream genes inducing Sxl only in XX female PGCs are in progress.

III. The role of heparan sulfate proteoglycans in JAK/ STAT signaling and distribution of its ligand, Unpaired.

The JAK/STAT pathway plays vital roles in development and homeostasis in animals. *Drosophila*, with its complete set of JAK/STAT components, provides a powerful genetic system to analyze the molecular functions of this essential pleiotropic pathway. In addition to its functions in embryonic, larval, and imaginal development, JAK/STAT signaling plays a critical role during several steps of *Drosophila* oogenesis. First, JAK activity is necessary in the somatic cells of the GSC niche to regulate production of the BMP signal that maintains GSCs. Furthermore, in the germarium, JAK signaling is required for maintenance and function of the supporting somatic escort cells. As cysts exit the germarium, JAK activity regulates the formation of stalk cells that separate developing egg chambers. Later in oogenesis, constant JAK activity is necessary for proper migration of the border cell cluster towards the posterior of the egg chamber. In ovaries with reduced JAK activity, there is a reduction in the number of border cells and stretched cells (terminal fates), and a concomitant increase in the number of main body cells. Furthermore, increased JAK activity from overexpression of the Janus kinase, Hop, or the Upd ligand causes cells to adopt terminal fates within the follicular epithelium. These results are consistent with the observed graded activation of JAK in the follicular epithelium and suggest that Upd acts as a morphogen during oogenesis. However, it has not been shown whether this

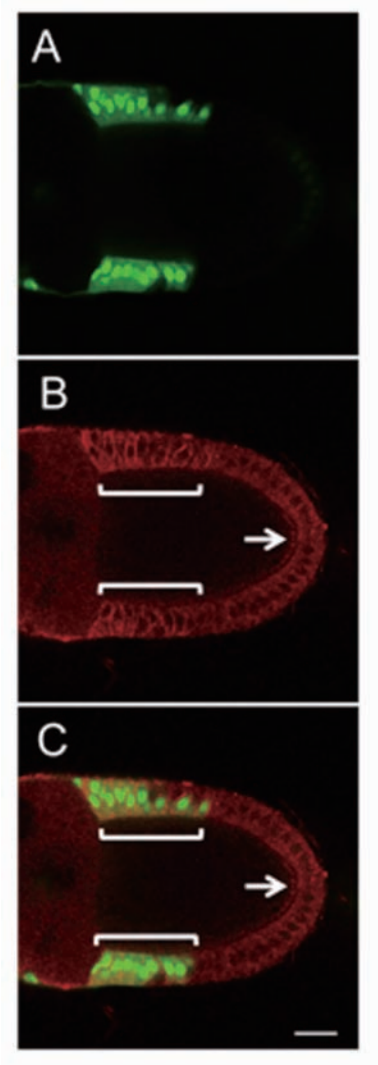

Figure 1. Dally regulates distribution of Upd.
(A-C) Distribution of Upd protein in *dally*-over expressed ovary. (A) *dally*-overexpressed clone (Green). (B) Upd protein distribution (Red). Upd is normally distributed at the apical surface of posterior follicle cells (Arrow). Upd accumulate on the cell surface of dally-over expressed clone (Brackets). (C) Merged image of A and B.

putative morphogen forms a concentration gradient that reflects the pattern of JAK activation.

Morphogens are important molecules in development and are defined by their ability to direct different cell fates over a distance in a concentration-dependent manner. It has been well established that extracellular signaling molecules of the Wingless/Wnt, Hedgehog (Hh), and Bone Morphogenetic Protein (BMP) families act as morphogens during *Drosophila* development. Despite extensive studies on the activities of these morphogens, it is not fully understood how these molecules generate and maintain their gradients in a tissue. A class of molecules that affect the gradient formation of all these morphogens is heparan sulfate proteoglycans (HSPGs). HSPGs are a family of carbohydrate-modified proteins abundantly found in the extracellular matrix and on the cell surface. Three families of HSPGs are widely conserved during animal evolution: syndecans, glypicans, and perlecans. In particular, two *Drosophila* HSPGs of the glypican family, *dally* and *dally-like protein* (*dlp*), have been shown to control BMP, Wnt, and Hh signaling. A previous study has shown that Upd protein expressed in cultured cells is tightly associated with the extracellular matrix and the addition of free heparin releases Upd into the medium. These observations suggested that Upd normally associates with HSPGs and thus may be regulated in a mechanism analogous to other morphogens.

We demonstrated that Upd indeed forms an extracellular gradient that activates JAK in a concentration dependent manner. As is the case for other secreted morphogens, Upd signaling was regulated by glypicans. Mutations in *dally* and *dlp* or in the HS biosynthetic enzymes, *sulfateless* (*sfl*) and *HS 2-O sulfotransferase* (*Hs2st*) led to aberrant JAK/STAT pathway activation and disruption of stalk cell specification. These alterations in JAK/STAT signaling and cell differentiation can be attributed to effects on the normal extracellular gradient of Upd by loss or changes in modification of the glypicans. Biochemical and histochemical studies showed that Dally and Upd physically bind to each other and co-localize on the surface of *Drosophila* S2 cells. In vivo, Upd accumulation on cells lacking glypicans was dramatically reduced, and reciprocally was enhanced upon ectopic expression of Dally (Figure 1). These results suggest that *Drosophila* glypicans serve to stabilize a novel morphogen, Upd, at the cell surface during oogenesis.

IV. The role of HSPGs in germline stem cell niche of *Drosophila*.

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 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 BMPs 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, the 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.

We identified *dally* and *dlp* as important components of the GSC niche in both sexes. In the female GSC niche, *dally* was expressed in niche cells. On the other hand, both of the glypicans were expressed in male GSC niche cells. Mutations for these glypicans caused a significant reduction in GSC number in both ovary and testis. In the *dally* mutant ovary, GSC lost appropriate activation of the signaling pathway by Dpp (a BMP homologue acting as a niche signal in female GSC niche). Conversely, ectopic expression of *dally* in female gonads caused an increase in GSC number with ectopic activation of the Dpp signaling pathway. These

results strongly suggest that *dally* defines the female GSC niche by regulating distribution of Dpp.

To address this, we have been trying hard to visualize Dpp distribution in the female GSC niche. By modifying protocols for antibody staining and generating new antibodies, we succeeded in visualization of Dpp distribution. We found that Dpp distribution was significantly expanded when *dally* was ectopically expressed in female gonads, while Dpp-producing cells were unaffected (Figure 2). The above results support our model that glypicans define the GSC niche by regulating extracellular distribution of niche signals.

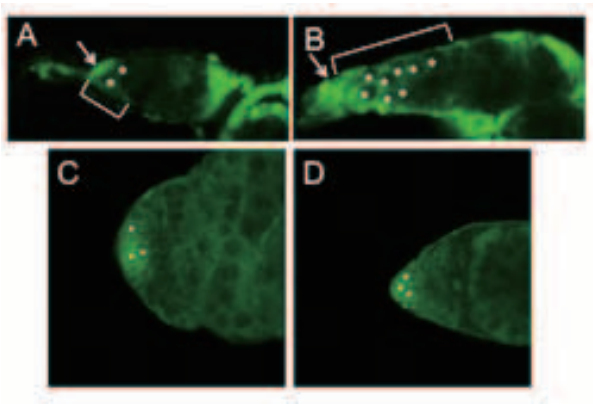

Figure 2. Distribution of GSC niche signals.
(A,B) Dpp distribution in distal tip region (germarium) of normal ovary (A) and of ovary expressing *dally* in the somatic cells throughout germarium (B). Green signal indicates Dpp protein (Brackets). Dpp distribution is expanded in *dally*-expressing ovary, compared to that observed in normal ovary. Arrows show niche cells, which are the source of Dpp. Asterisks indicate GSCs. (C, D) Distribution of male GSC niche signals, Upd (Green, C) and Gbb (Green, D). Distribution of both niche signal was limited within male GSC niche. Asterisks indicate GSCs.

We also investigated molecular function of glypicans in the male GSC niche. We found that *dlp* is required for signaling pathway by Gbb (the other BMP homologue acting as a niche signal in male GSC niche). Upd is also known as male GSC niche signal. Since our data showed that *dally* regulates the morphogen gradient of Upd (see section III), we speculate that *dally* and *dlp* separately regulate Upd and Gbb distribution in the male GSC niche, respectively. For the first step to address this possibility, we tried to visualize the Upd and Gbb distribution in the male GSC niche. We found that Upd and Gbb were both enriched in the male GSC niche (Figure 2). By utilizing this system, we are now investigating whether these glypicans define the male GSC niche via regulating distribution of niche signals.

Publication List

〔Original papers〕

- Hayashi, Y., Sexton, T.R., Dejima, K., Perry, D.W., Takemura, M., Kobayashi, S., Nakato, H., and Harrison, D.A. (2012). Glypicans regulate JAK/ STAT signaling and distribution of the Unpaired morphogen. Development *139*, 4162-4171.
- Nishimiya-Fujisawa, C., and Kobayashi, S. (2012). Germline stem cells and sex determination in Hydra. Int. J. Dev. Biol. 56, 499-508.
- Ohhara, Y., Kayashima, Y., Hayashi, Y., Kobayashi, S., and Yamakawa-Kobayashi, K. (2012). Expression of beta-adrenergic-like octopamine receptors during Drosophila development. Zool. Sci., *29*, 83-89.