DIVISION OF DEVELOPMENTAL GENETICS †

Professor
KOBAYASHI, Satoru

Assistant Professor:	*HAYASHI, Yoshiki*
	SATO, Masanao
Technical Staff:	*NODA, Chiyo*
NIBB Research Fellow:	*OTA, Ryoma*
Postdoctoral Fellow:	*FUJISAWA, Chiemi*
	OHARA, Yuya
	ASAOKA, Miho
SOKENDAI Graduate Student:	*SUGIYAMA, Arisa*
	SHINOZUKA, Yuko
	SUGIMORI, Seiko
	MORITA, Shunpei
Technical Assistant:	*SATO, Kaori*
	YAMAMOTO, Manami
	WASHIO, Midori
	KAWAMOTO, Yuri
Secretary:	*HONDA, Satoko*

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, GSC niche function, and metamorphosis 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 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 primordial germ cells (PGCs) during embryogenesis. To understand its function, we over-expressed the Ovo-A repressor only in the 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.

While identifying the downstream genes regulated by Ovo-B in germline, we found that Ovo is required to induce germline-enriched genes, and conversely, it represses somatically-expressed genes in PGCs. Thus, we speculate that maternal Ovo has an important role in germline-fate determination. Collaboration work is now on-going to clarify the function of the *ovo* gene in mouse germline development.

II. 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 the GSC niche.

To address this question, we have been investigating the function of Heparan Sulfate Proteoglycans (HSPGs) in the GSC niche. HSPGs are an evolutionally conserved family of sugar modified proteins, which are an essential component of the extracellular matrix. One of the important functions of HSPGs during animal development is to regulate distribution of growth factors in extracellular space by binding to them. Thus, we speculated that HSPGs could retain a sufficient amount of niche signals for GSC maintenance. We found that Glypcan, a membrane-associating type of HSPG, is an essential component of the GSC niche both in female and male gonads. Glypican was highly expressed in niche cells both in ovary and testis, and its mutations caused a significant reduction in GSC number. In the GSC of the mutant ovary, the signaling pathway activated by Dpp (a BMP homologue acting as a niche signal) was impaired. Conversely, ectopic expression of Glypican in female gonads caused an increase in the number of GSCs with Dpp signaling. These results strongly suggest that Glypican defines the female GSC niche by regulating distribution of Dpp.

The question of whether the other HSPGs have functions in the GSC niche remains unclear. Since disrupting biosynthesis of all HSPGs by knockdown of the *NDST* gene caused a more severe GSC-loss phenotype than the Glypican mutant, the other types of HSPGs could have functions in the GSC

†: This laboratory was closed on 31 March, 2015.

niche. We found that Syndecan and Perlecan, two evolutionally conserved groups of HSPGs, are essential in the GSC niche. These HSPGs were highly expressed in female GSC niche cells, and reduction of their function in niche cells caused a decrease in GSC number. We further found that, in the ovaries with reduced Perlecan function, ectopic GSC-like cells were also observed. This phenotype has not been observed in Glypican mutants. Thus, we speculated that Perlecan could regulate Dpp distribution in the GSC niche, in a way distinct from Glypican. We have succeeded in visualizing Dpp protein distribution in the female GSC niche. When Glypican was ectopically expressed in female gonads, Dpp distribution was ectopically observed in Syndecan and Perlecan mutant ovaries. Furthermore, we have also succeeded in visualizing the GSC niche signal in male gonads. This enables us to study HSPG function in the male GSC niche.

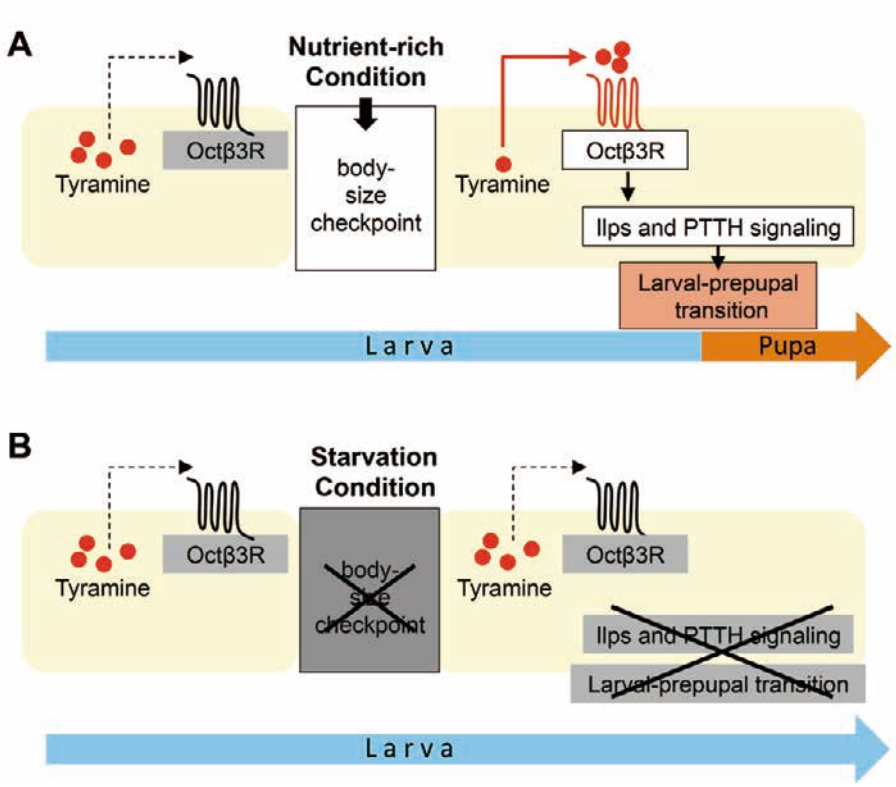

Figure 1. Our model explaining the regulation of metamorphosis by Octβ3R
(A) Before the attainment of body-size checkpoint, tyramine is stored in PG cells, so as not to activate Octβ3R. Once larvae have attained body-size checkpoint under nutrient-rich conditions, tyramine is secreted from the PG to activate Octβ3R signaling in an autocrine manner, leading to the larval–prepupal transition via the Ilps and PTTH signaling pathways. (B) When larvae fail to attain body-size checkpoint under a starvation condition, tyramine remains unsecreted from the PG; consequently, the Octβ3R, Ilps, and PTTH signaling pathways fail to be activated, resulting in arrest at the larval–prepupal transition.

III. The role of β3-octopamine receptor in the developmental transition from larvae to reproductive adults

The developmental transition is a well-known biological process in which the organism alters its body morphology in order to proceed from the juvenile stage to the adult reproductive stage. How this processe is precisely regulated in response to developmental and environmental cues is a longstanding question in biology.

In holometabolous insects, the steroid hormone ecdysone plays a pivotal role in metamorphosis. In *Drosophila*, ecdysone is produced in the prothoracic gland (PG) and then converted into its active form, 20-hydroxyecdysone (20E), in the peripheral organs. The activities of 20E terminate larval development and growth and initiate metamorphosis. Ecdysone biosynthesis is regulated in the PG by neuropeptides, enabling modulation of the timing of 20E pulses during development. The stimulator of ecdysone biosynthesis is prothoracicotropic hormone (PTTH) and Insulin-like peptides (Ilps), which activate the production of ecdysone biosynthetic proteins. In addition to these neuropeptides, the larval–prepupal transition is modulated by environmental cues such as nutritional conditions that influence larval body size. For example, early third-instar larvae attain the body-size checkpoint required for the transit from larva to prepupa. Although the checkpoint is believed to ultimately modulate ecdysone production in the PG, its downstream effectors and signaling pathway remain elusive.

We found that monoaminergic autocrine regulation of ecdysone biosynthesis in the PG is essential for metamorphosis. PG-specific knockdown of *Octβ3R*, resulted in arrested metamorphosis due to lack of ecdysone. Knockdown of tyramine biosynthesis genes expressed in the PG caused similar defects in ecdysone production and metamorphosis. Moreover, PTTH and Ilps signaling were impaired by *Octβ3R* knockdown in the PG, and activation of these signaling pathways rescued the defect in metamorphosis. Thus, monoaminergic autocrine signaling in the PG regulates ecdysone biogenesis in a coordinated fashion upon activation by PTTH and Ilps. We propose that monoaminergic autocrine signaling acts downstream of a body-size checkpoint that allows metamorphosis to occur when nutrients are sufficiently abundant.

Publication List

〔Original papers〕

● Chanut-Delalande, H., Hashimoto, Y., Pélissier-Monier, A., Spokony, R., Dib, A., Kondo, T., Bohère, J., Niimi, K., Latapie, Y., Inagaki, S., Dubois, L., Valenti, P., Polesello, C., Kobayashi, S., Moussian, B., White, K., Plaza, S., Kageyama, Y., and Payre, F. (2014). Pri peptides are mediators of ecdysone for the temporal control of development.

Nature Cell Biol. 16, 1035-1044.
- Hayashi, M., Sato, M., Iwasaki, Y., Onozawa, T., Katayama, N., Nagasaka, Y., Sadaie, S., Kobayashi, S., and Yoshizaki, G. (2014) Enrichment of spermatogonial stem cells using side population in teleost. Biol. Reprod. 91, 1-8.
- Lim, R., Anand, A., Nishimiya-Fujisawa, C., Kobayashi, S., and Kai, T. (2014). Analysis of Hydra PIWI proteins and piRNAs uncover early evolutionary origins of the piRNA pathway. Dev. Biol. 386, 237-251.
- Nishimura, T., Herpin, A., Kimura, T., Hara, I., Kawasaki, T., Nakamura, S., Yamamoto, Y., Saito, T., Yoshimura, J., Morishita, S., Tsukahara, T., Kobayashi, S., Naruse, K., Shigenobu, S., Sakai, N., Schartl, M., and Tanaka, M. (2014). Analysis of a novel gene, Sdgc, reveals sex chromosome–dependent differences of medaka germ cells prior to gonad formation. Development 141, 3363-3369.