DIVISION OF DEVELOPMENTAL GENETICS

Professor
KOBAYASHI, Satoru

Assistant Professors:	*HAYASHI, Yoshiki*
	SATO, Masanao
Assistant Professor (Specially Appointed):	
	KAGEYAMA, Yuji
Technical Staff:	*NODA, Chiyo*
NIBB Research Fellow:	*HASHIYAMA, Kazuya*
Postdoctoral Fellows:	*KUMATA, Yuji*
	FUJISAWA, Chiemi
	INAGAKI, Sachi
	*KAGEYAMA, Yuji**
	HASHIMOTO, Yoshiko
Graduate Students:	*KUBO Satoru*
	SHINOZUKA Yuko
Visiting Scientists:	*FUJISAWA Toshitaka*
	OHARA Yuya
Technical Assistants:	*SATO, Kaori*
	ISHIHARA Hitomi
	NIIMI Kaori
	OKUDAIRA, Yuichi
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, germline sex determination and GSC niche formation 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

"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 primordial germ cells (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. Contrary to prevailing dogma, we found in *Drosophila* that *Sex lethal* (*Sxl*) is expressed in female pole cells before gonad formation and acts autonomously in these cells to induce female development.

Sxl is transiently expressed in 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. Our findings provide powerful evidence for *Sxl* as a master gene that directs a female germline fate. XX pole cells initiate female sexual identity based on their *Sxl*

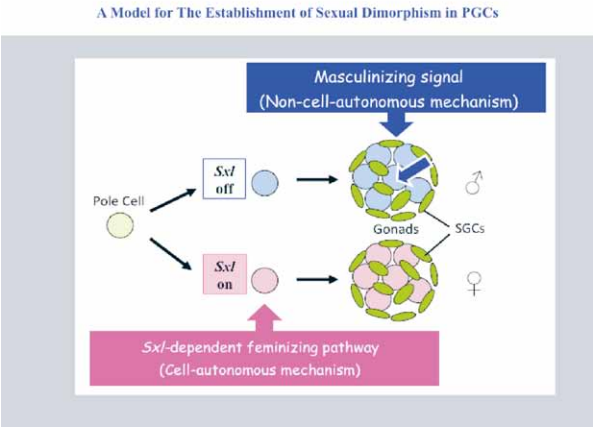

Figure 1. A model for the regulation of sexual dimorphism in pole cells.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

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.

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 (Figure 2). 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 (Kitadate and Kobayashi, 2007).

We further found that Notch signaling induces hub differentiation (Kitadate and Kobayashi, 2010) (Figure 3). 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.

We showed that epidermal growth factor receptor (Egfr) is activated in the posterior SGCs to repress hub differentiation (Figure 4). 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 (Kitadate and Kobayashi, 2010).

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, 3 and 4). 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 through signaling from PGCs to SGCs to modulate niche size, and this serves as a mechanism securing GSCs.

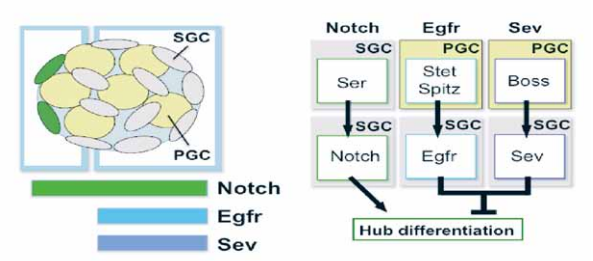

Figure 2. Hub (green) differentiation is controlled by negative regulators, Sev and Egfr and a positive regulator, Notch.

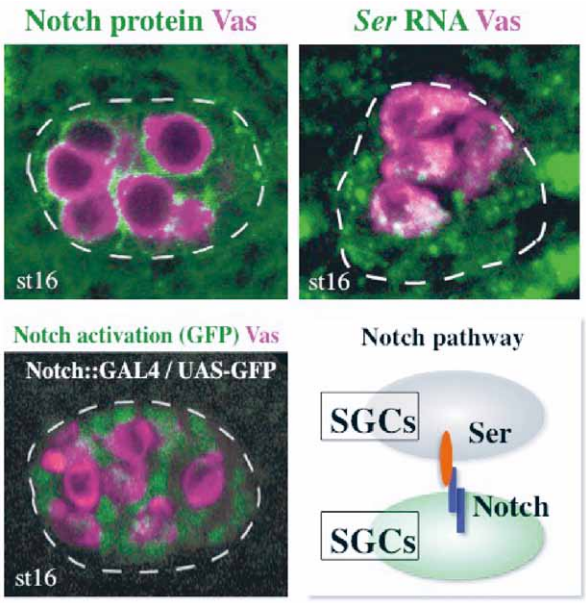

Figure 3. Expression of Notch and *Serrate* in male embryonic gonads.

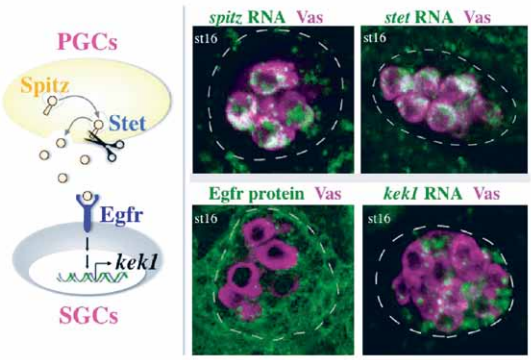

Figure 4. Expression of Egfr signaling components in male embryonic gonads.

IV. Studies on short ORF-containing transcripts in Drosophil

Transcriptome analyses in eukaryotes, including mice and humans, have identified many poly(A)-containing transcripts that only contain short ORFs (sORFs; less than 100 aa). These sORF transcripts are believed to most likely function

as non-coding RNAs (ncRNAs), but growing evidence strongly suggests that a substantial proportion of these "non-coding" transcripts are actually translated into tiny peptides.

We have reported that the *polished rice* gene polycistronically encodes extremely small peptides (11 or 32 aa residues) and regulates epidermal development in *Drosophila*. *pri* is essential for the formation of specific F-actin bundles that prefigure epithelial cellular processes, or trichomes. This year, we have been focusing on genetic interaction of *pri* and *shavenbaby* (*svb*), the master regulator of trichome formation. SVB protein is a unique transcription factor that contains both a transcriptional repression domain and an activation domain, as well as a DNA binding signature of C_2H_2-type zinc-finger. Analysis of gene expression profiles of *pri* mutant flies demonstrated that *pri* is required for activation of *svb* target genes (Kondo et al., 2010) (Figure 5). Transcription assay using an SVB-responsive reporter showed that SVB protein functions as a transcriptional repressor in the absence of *pri* products, while co-expression of *pri* results in activation of the reporter, suggesting that transcriptional activity of SVB protein depends on *pri* activity. Coincidently, in cultured cells and in living embryos, sub-nuclear localization of SVB was changed upon *pri* expression: SVB was detected in a punctate pattern in the absence of *pri* while being more diffused in the presence of *pri*. Biochemical analysis showed that *pri* induced N-terminal truncation of SVB, which results in loss of the repression domain. Truncation is proteolytic, because 1) *svb* mRNA is not modified by *pri* expression, 2) known start codons used in Drosophila do not match to the N-terminus of truncated SVB protein, 3) amino acid sequence, but not DNA sequence, of the truncation site is highly conserved in *Drosophila* species. Taken together, we conclude that *pri* converts SVB from a transcriptional repressor to an activator. These results demonstrate that sORF genes play important roles in *Drosophila* and further analysis of sORF genes should elucidate unexplored novel genome functions of eukaryotes.

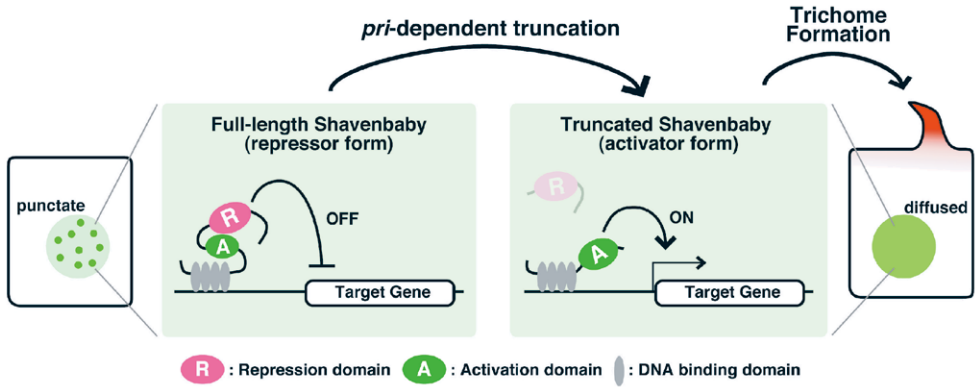

Figure 5. Biological roles of the *polished rice* gene. During trichome formation, *pri* induces truncation of SVB protein, converting transcriptional activity of the protein. Accompanied by activity conversion, subnuclear localization of SVB is also drastically changed. The truncated form of SVB protein in turn activates transcription of target genes, which results in trichome formation.

Publication List

〔Original papers〕

- Kitadate, Y., and Kobayashi, S. (2010). Notch and Egfr signaling act antagonistically to regulate germline stem cell niche formation in *Drosophila* male embryonic gonads. Proc. Natl. Acad. Sci. USA *107*, 14241-14246.
- Kondo, T., Plaza, S., Zanet, J., Benrabah, E., Valenti, P., Hashimoto, Y., Kobayashi, S., Payre, F., and Kageyama, Y. (2010). Small peptides switch the transcriptional activity of Shavenbaby during *Drosophila* embryogenesis. Science *329*, 336-339.
- Niwa, R., Ito, K., Namiki, T., Shimada-Niwa, Y., Kiuchi, M., Kawaoka, S., Kayukawa, T., Banno, Y., Fujimoto, Y., Shigenobu, S., Kobayashi, S., Shimada, T., Katsuma, S., and Shinoda, T. (2010). *Non-molting glossy/shroud* encodes a short-chain dehydrogenase/reductase that functions in the "Black Box" of the ecdysteroid biosynthesis pathway. Development *137*, 1991-1999.