LABORATORY OF MOLECULAR GENETICS FOR REPRODUCTION

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Laboratory Scope

Sexually dimorphic gonads mainly consist of two different cell lineages, somatic gonadal cells and germ cells. During the course of development, the gonadal precursor cells should be specified through the process of mesoderm patterning, which subsequently associates primordial germ cells to form the indifferent gonad. Once indifferent gonad is formed in the gonadal area, sex determination gene is expressed in the gonadal mesoderm and organogenesis of the gonads (sex differentiation) initiates in mice.

Since the sex determination gene in mice was identified in 1990, much research has been intensively focused on the testis development. When considering, however, the differentiation of the precursor cells determining sex, the interaction of somatic cells and germ cells, the differentiation of primordial germ cells (PGCs) to germ cells having the capability to undergo meiosis, and so on, there remain many important and fascinating problems to be solved.

Our laboratory aims to reveal the molecular mechanisms of the formation of gonads and the sex differentiation, with an emphasis on visualizing specific cell lineages in living medaka embryos and by applying molecular genetics to medaka embryos.

I. Analysis of movement of primordial germ cells by timelapse movies

One of the advantages using medaka is that the embryos are transparent and the development can be seen from outside. This encouraged us to generate transgenic medaka exhibiting GFP exclusively in germline and succesfully established the trangenic lines (*olvas*-transgenic medaka). On the other hand, we have been screening medaka genes expressing in germline and found that some of the 3'UTRs are critical for maintenance and translation of their own transcripts specifically in germline. This translational mechanism also allows to visualize the germline by injecting GFP-3'UTR chimeric RNA into fertilized eggs.

With the two means mentioned above, we have been monitoring the origin and the mode of movement of medaka primordial germ cells (PGCs) during embryogenesis. We revealed that presumptive PGCs are already present by early gastrulation stage by analyzing the expression of one of the germline-expressing genes and that PGCs exhibit three different modes of migration to reach the gonadal area. After appearance of PGCs at early gastrulation stage around the animal pole, timelapse analyses show that PGCs actively move between epiblasts and hypoblasts towards the marginal region (Figure 1). This movement is dependent on the activity of the chemo-attractant, CXCR4 (the first mode). PGCs then move medially towards the embryonic axis with lateral somatic cells undergoing convergent movement (the second mode). After the bilateral alignment of PGCs along the embryonic axis, the PGCs actively resume directional movement towards the region of a gonadal field in the posterior lateral plate mesoderm. SDF-1a and HMGCoA reductase are independently involved in this posterior migration (the third mode) (Kurokawa *et al*. 2006).

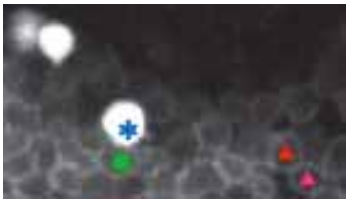

Figure 1. PGCs (asterisk) migrate between epiblasts (square) and hypoblasts (triangles) during epiboly.

II. Origin of gonadal mesoderm

Much research has focused on the mechanisms of sex differentiation. However, the origin and the lineages of the gonadal precursor cells have remained to be elucidated in vertebrates.

We have successfully demonstrated the presence of the gonadal field where the precursors of gonadal mesoderm arise. This region is located at the most posterior end of *sdf-1a* expression domain. It is known that *sdf-1a* functions as a guidance cue for PGCs to migrate to the gonadal area and the expression becomes restricted to the posterior lateral mesoderm. Our result indicates that the most posterior end of *sdf-1a* expression domain is the place that coordinates PGC migration and development of the gonadal mesoderm. We further demonstrated that

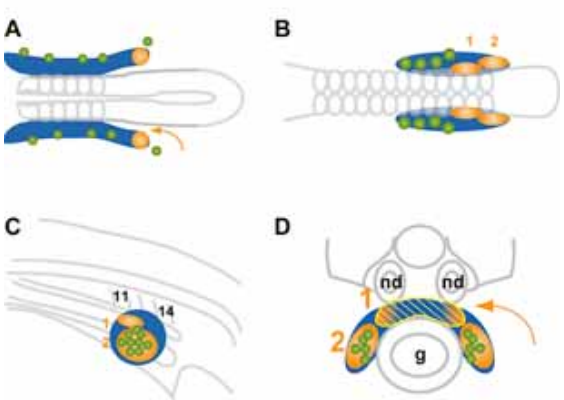

Figure 2. The two different precursors of gonadal mesoderm arise from the gonadal field (orange). *sdf-1a* expression domain (blue). PGCs (green).

two different populations with distinct gene expression are spatially organized from the gonadal field along the embryonic axis and are specified before the gonadal primordium forms (before the sex is determined) (Nakamura *et al*. 2006) (Figure 2).

Generally, in vertebrates, the presence and characteristics of gonadal precursors before sex determination are poorly understood. Cellular interaction among the different cell precursors, which brings about development of the supporting cells such as Sertoli cells and granulosa cells, also remains to be elucidated.

We have generated medaka embryos that completely lack germ cells in the gonadal primordium by impairment of PGC migration and have confirmed the biased ratio of phenotypic sex in the germ cell-less adult medaka. The morphology of the gonad in the germ cell-less medaka exhibits the appearance of neither testis nor ovary. We are currently investigating the involvement of germ cells in the development of the gonadal supporting cells.

III. Generation of transgenic medaka visualizing different cell lineages of the supporting cells in the gonad

To clarify how many kinds of supporting cells appear during the formation of gonadal primordium and the sex differentiation, we are systematically identifying the different types of supporting cells by *in situ* hybridization and generating the transgenic medaka that visualizes each type of the supporting cells. Currently we are trying to monitor different cells with GFP fluorescence driven by the regulatory regions of several different genes. One line has been established as a transgenic line that allows us to observe somatic cells in gonadal primordium (Figure 3). The transgenic medaka suggests that several

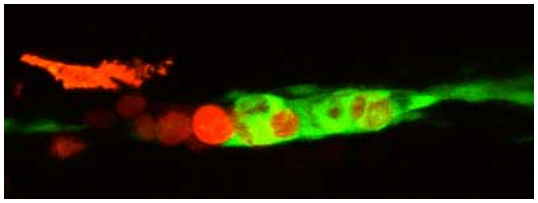

Figure 3. Somatic cells (green) surround germ cells (red) in the gonadal primordium (from in *vivo* timelapse movie).

different types of supporting cells have already been differentiated before sexual differentiation (organogenesis of ovary or testis) occurs.

The attempt to monitor the process of development of each lineage has also been made in living embryos and larva by timelapse movies. In order to solve the difficulties in visualizing the cells located in the deep positions in the embryos and larva, the confocal microscopy and SPIM have been applied to the transgenic embryos and larva. This attempt is still in progress in collaboration with Jochen Wittbrodt Lab. in EMBL.

IV. Analysis of mutant medaka affecting formation of gonad

Medaka is a small vertebrate and produces next generations in 3 months after hatching. These characteristics are suitable for applying molecular genetics to this small animal.

In collaboration with ERATO Kondoh differentiation signaling project, we have been screening mutants affecting the development of primordial germ cells and the formation of gonads. The screening has been performed in such a way that particular attention is paid to the presence, the number or the distribution pattern of germ cells at a somitogenesis stage and at ten days post hatching (10 dph). Nine mutants (19 alleles) and twelve mutants (14 alleles) were identified for PGCs and gonads, respectively.

One mutant, *totoro*, is very interesting in that the phenotype has not been described before in other animals. *totoro* has a swollen large abdomen filled with a gonad. Our analyses show that the phenotype is semidominantly inherited. Gonads are sex-reversed in genetic males and fullfilled with numerous oocytes regardless of its genetic sex. The positional cloning is ongoing with the ERATO project and three genes are identified as the possible candidate genes responsible for the phenotype.

Figure 4. Blue staining shows PGCs in the gonad. Left: *zenzai* mutant that cannot maintain germ cells. Middle: wild type. Right: *totoro* mutant that shows overproliferation of germ cells.

Another mutant, *zenzai*, is a good contrast with the *totoro* mutant and is unique in that germ cells are not maintained in the gonad (Figure 4). Inheritance of the phenotype indicates that the allele is recessive. The characterization and the positional cloning are also in progress. We are narrowing down the genomic region responsible for *zenzai* phenotype to approximately 110 Kb.

We are also characterizing other mutants in another category, irregular distribution of germ cells in gonads. These mutants include *hadare*, *mizore*, *hyou* and *arare*.

Publication List:

Original paper

Hano, T., Oshima, Y., Oe, T., Kinoshita, M., Tanaka, M., Wakamatsu, Y., Ozato, K., and Honjo, H. (2005). Quantitiative Bio-imaging analysis for evaluation of sexual differentiation in germ cells of *olvas*-GFP/STII YI medaka (*Oryzias latipes*) nanoinjected in ovo with ethinylestradiol. Environ. Toxicol. Chem. *24*, 70-77.