LABORATORY OF MOLECULAR GENETICS FOR REPRODUCTION

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Laboratory Scope

Our laboratory aims to reveal the molecular mechanisms of formation of the gonads and sex differentiation. We are using medaka fish (*Oryzias latipes*) for these purposes.

Medaka has been recently established as a model vertebrate. The entire genome sequence was determined and a variety of inbred strains with a large polymorphic genome is available, which allows us to investigate biological phenomena by the means of molecular genetics. In addition, an exogenous gene can be introduced into medaka genome (transgenic medaka) and cells can be transplanted to host medaka to generate chimera medaka.

With these advantages, we have been generating transgenic medaka enabling us to identify the different cell lineages by fluorescence and to analyze the process of gonad formation and sex differentiation in vivo. Additionally, in order to identify the genes essential for gonadogenesis, we carried out a mutational screening of medaka with a defect in gonads and are performing a positional cloning. With these two unique analytical methods (visualising cells and mutants), we are attempting to unveil the fundamental mechanisms of sex differentation which are believed to be common to many organisms.

I. Identification of the gonadal fields that coordinate germ cell migration and development of gonadal somatic cells

Much research has focused on the function of the genes involved in sex differentiation using gene-disrupted mice. However, the functions of each cell type and the interactions between the different cell lineages are totally unknown during sex differentiation. It is important to understand the origin and the lineages of the gonadal precursor cells in order to analyze cellular events.

Since one of the important cell lineages is germline, we first characterized the migration of primordial germ cells (PGCs) towards the gonadal area. We found three different modes of PGC movement: 1) active migration towards the peripheral region at early gastrulation (*cxcr4*-dependent), 2) passive movement towards the embryonic body with convergent movement of somatic cells at early segmentation stages, and 3) active posterior migration towards the most posterior end of *sdf1a*

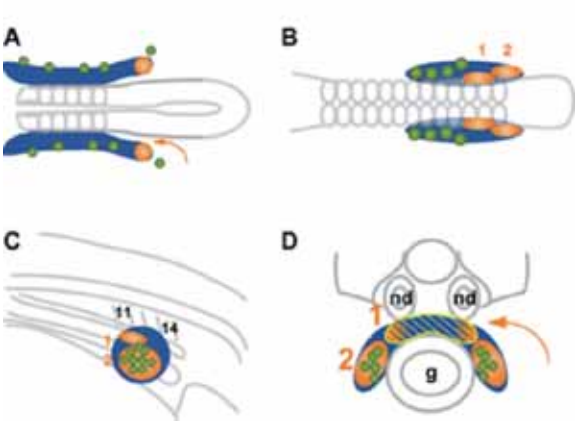

Figure 1. The two different precursors of gonadal mesoderm arise from the gonadal fields (orange). *sdf-1a* expression domain (blue). PGCs (green) (From reference, Nakamura et al, 2006).

expression domain at late segmentation stages (*sdf1a*-dependent) (Kurokawa *et al*, 2006). We characterized the most posterior region of *sdf1a* expression domain and showed that it has the same properties as gonadal fields where the precursors of gonadal mesoderm arise. This result indicates that the gonadal field is the place that coordinates PGC migration and the development of the gonadal mesoderm. We further demonstrated that two different populations with distinct gene expression are spatially organized from the gonadal precursors along the embryonic axis and are specified before the gonadal primordium forms (i.e., before the sex is determined) (Nakamura *et al*. 2006) (Figure 1).

II. Germ cells are essential for sexual dimorphism of the gonads

The nature of somatic cells and germ cells is a topic of broad and long standing interest. Many studies have therefore explored the interaction between germ cells and

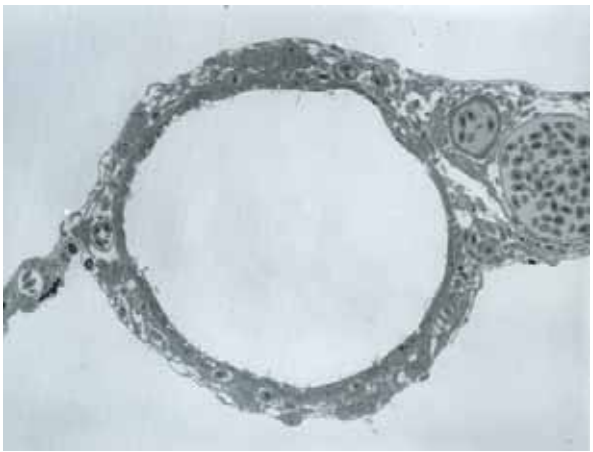

Figure 2. The germ cell-deficient gonad exhibits a single fundamental structure common to both ovary and testis. A large lumen is located in the middle and a single layer of supporting like cells encloses the lumen, which is separated by a basement membrane from an outer stromal region.

gonadal somatic cells. The dogma resulting from these studies is that germ cells do not significantly affect the sex differentiation of gonads.

We generated medaka embryos that completely lack germ cells in the gonadal primordium by impairment of PGC migration and found female to male sex reversal 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 (Figure 2). Novel *aromatase*-expressing theca cells that we identified in ovary did not develop properly and female supporting cells that control gametogenesis began to express male-specific genes as development of the gonad proceeded, suggesting trandifferentiation from female to male supporting cells. The cells producing male sex steroid hormone persisted in the germ cell-deficient medaka. All of this data indicated that the gonadal somatic cells are predisposed to adopting male development and the production of male steroid hormone results in sex reversal to male secondary sex characteristics. Thus, contrary to accepted dogma, we demonstrated that germ cells are essential for sexually dimorphic gonads (Kurokawa *et al.* submitted).

III. Generation of transgenic medaka to identify the cell lineages that constitute the gonads

To clarify the cell types that constitute the gonad, we are generating transgenic fish to visualize the cell lineages. We have established several lines of transgenic fish that allow us to analyze how they build up the gonad during the course of development. Crossing the transgenic fish

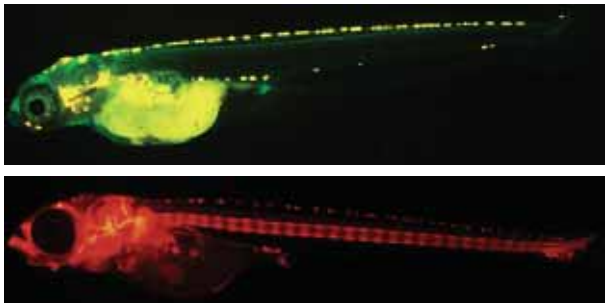

Figure 3. Different colored transgenics allowed us to reconstruct gonad structures composed of different cell types and to monitor cell movement in the structures.

with different colors (Figure 3) enabled us to successfully reconstitute novel units of structure that have not been reported yet in the gonad.

An attempt to monitor the process of development of each lineage has also been made in living embryos and larva using timelapse movies. In order to solve the difficulties in visualizing the cells located in the deep positions in the embryos and larva, 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. Identification of the genes of medaka mutants with defects in the gonads

In collaboration with SORST Kondoh team, 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 and 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 of particular interest because of the excessive number of germ cells that are arrested in early development of follicle growth and because male to female sex reversal occurs irrespective of their genetic sex. As a result of the positional cloning, we have identified a candidate gene and are now proving that mutation in the gene is responsible for all the phenotype of *totoro* (Morinaga *et al*, submitted).

Figure 4. Blue staining shows PGCs in the gonad. Left: *zenzai* mutant that cannot maintain gerrn 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. We again identified one possible candidate gene for the phenotype of *zenzai* mutant.

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

Publication List:

Original papers

Kurokawa, H., Aoki, Y., Nakamura, S., Ebe, Y., Kobayashi, D., and Tanaka, M. (2006). Time-lapse analysis reveals different modes of primordial germ cell migration in the medaka *Oryzias latipes*. Develop. Growth Differ. *48*, 209-221.

Nakamura, S., Kobayashi, D., Aoki, Y., Yokoi, H., Ebe, Y., Wittbrodt, J., and Tanaka, M. (2006). Identification and lineage tracing of two population of somatic gonadal precursors in medaka embryos. Dev. Biol. *295*, 678-688.

Saito, T., Fujimoto, T., Maekawa, S., Inoue, K., Tanaka, M., Arai, K., and Yamaha, E. (2006). Visualization of primordial germ cells in vivo using. GFP-*nos*1 3íUTR mRNA. Int. J. Dev. Biol. *50*, 691-700.