LABORATORY OF MOLECULAR GENETICS FOR REPRODUCTION

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Laboratory Scope

Our laboratory aims to reveal the molecular mechanisms of the formation of the gonads and sex differentiation. We use medaka fish (*Oryzias latipes*) for these purposes and have been generating transgenic medaka (Figure 1) 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 differentiation and plasticity common to many organisms.

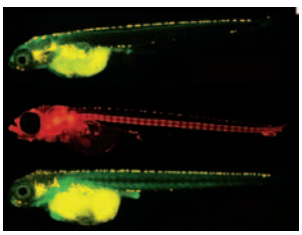

Figure 1. Various Transgenic medaka.

Ⅰ. Balancing between germ cells and gonadal somatic cells is important for sex differentiation of gonads.

We have generated medaka that completely lack germ cells in the gonad by impairment of PGC migration. These medaka reveal two important aspects of germ cell functions on the sex differentiation of gonads. Firstly, the morphological structure of germ cell-less gonads indicates the importance of germ cells for the formation of sex-specific structures in the gonads, possibly organizing the common unit into sex-specific structures dependent on its genetic sex. The second point is that germ cells are essential for development of the ovary. Without germ cells, both genetical female and male medaka exhibit male secondary sex characteristics and gonadal somatic cells are completely masculinized. These results suggest that the female character of germ cells antagonizes the autonomous masculinization of gonadal somatic cells and that balancing between germ cells and gonadal somatic cells is essential for both sex differentiation and maintenance of sex.

In support of this claim, medaka mutants that display a germ cell-hypertrophic phenotype, *hotei*, exhibit female secondary sex characteristics. The gene responsible for this phenotype is the type II receptor gene for anti-Müllerian homone (*amhrII*). We found that both *amhrII* and its ligand, *amh*, are expressed in gonadal somatic cells but not in germ cells.

In order to understand if male to female sex reversal in *hotei* mutants occurs because of a gonadal somatic cell-autonomous defect or because of a large number of germ cells, we have generated germ cell-deficient *hotei* mutants. None of the germ cell-less *hotei* mutants show any sex reversal of secondary sex characteristics. In addition, female-specific gene expression that can be seen in genetic male *hotei* mutants is also abolished when the germ cells are depleted. These results indicate that feminization of the gonad is a consequence of hypertrophic germ cells but not of a gonadal somatic cell-autonomous event.

The results from both germ cell-deficient medaka and germ cell-hypertrophic mutants (*hotei*) reveal an intrinsic mechanism of sex differentiation that is independent of genetic sex determination. We propose that, irrespective of genetic sex, germ cells have an intrinsic character that canalizes feminization of the gonad while gonadal somatic cells are predisposed to male development. According to this proposal, the function of the medaka testis determination gene on the Y chromosome can be explained as an enhancement of masculization that conquers canalization towards ovary by germ cells. The balancing of the two opposing characters, canalization of germ cells towards feminization and predisposition of gonadal somatic cells towards masculinization, may be a conserved cellular interaction among vertebrates. In fact, there have been several reports of the masculization of mice ovaries following the depletion of germ cells by genetic or physical manipulations. Even in an undifferentiated gonocolist, zebrafish, which firstly develops ovaries before some of the population turns into males, prior to the development of testis the germ cell number decreases once by apoptosis. The suppression of the germ cell number at the early stage of medaka gonadal sex differentiation and the decrease in germ cells in zebrafish can be viewed as different ways of achieving the same result (Tanaka et al., 2008 DGD: Saito et al., Sex. Dev. in press).

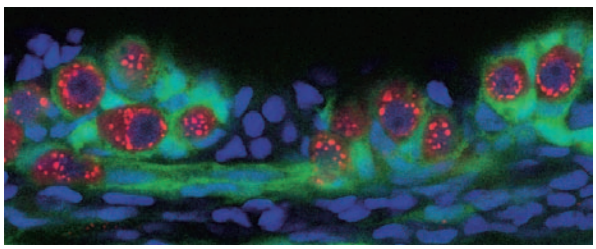

Figure 2. A right lobe of *sox9bp:GFP*-transgenic medaka. Blue: DAPI, Red: Germ Cell and Green: GFP.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

Ⅱ. Gonadal morphology during early stages of sex differentiation

Our previous results indicated that, prior to the formation of the gonadal primordium, two different precursors of gonadal somatic cells are established in the most posterior region of lateral plate mesoderm. These precursors express either *sox9b* or *ftz-f1*. In mammals, *sox9b* is specifically expressed in the male supporting cells surrounding germ cells (Sertoli cells and their precursors) and is known as the gene indispensible for testis formation. In order to understand the types of cells arising from the precursors in medaka gonad, we have generated transgenic medaka that allow us to keep track of *sox9b*-expressing cells in the gonad.

With the formation of the gonadal primordium, *sox9b*-expressing cells ensheathed primordial germ cells. Obervation of *sox9b*-expressing cells revealed the change of the morphology of the primordium. The gonadal primordium firstly forms as a single structure located along the midline. The cells that do not express *sox9b* appear and invade in the middle of the single gonadal primordium. As a consequence, the gonadal primordium splits into bilateral lobes. This process occurs soon after the single gonadal primorium forms (Figure 2).

Sox9b-expressing cells in male gonads keep surrounding the germ cells and begin to express the male supporting cell (Sertoli) marker, *dmrt1*, which is similar to those in mammalian testis development. In female gonads, the germ cells that undergo synchronous division enter meiosis and form follicles. Some of the population of germ cells in female gonads enters meiosis and follicles are formed. Unlike mammals, expression of *sox9b* is retained and found in the cells surrounding germ cells. Very interestingly, the granulosa cells of small follicles (primordial follicles) also express *sox9b*, but its expression diminishes immediately with the progress of folliculogenesis and the onset of the expression of the granulosa cell marker, *foxl2*.

Collectively, the observations using *sox9b*-transgenic medaka strongly suggest that the supporting cells expressing *sox9b* in the gonadal primordium are present as precursors of both Sertoli cells and granulosa cells.

Ⅲ. Germ cell development with change of the localization of germ-granule components

Germ granules are germ cell-specific intracellular structures and are essential for germ cell development. An examination of their components - olvas (vasa), nanos and tdrd1 (tudor) - reveals that they alter their localization in the cytoplasm during the early stage of sex differentiation of the gonads. By immunohistochemical analysis, these three germline-specific proteins were detectable on granule-like structures in the cytoplasm of migrating primordial germ cells (PGCs). In the germ cells of the gonadal primordia, these granules formed a hollow area lacking these three protein components. During the sexual differentiation of the female gonads, the granules were found to be reduced in size in the germ cells undergoing cystic division and they showed perinuclear localization in the oocytes. The germ cells in the male gonads, however, retained their hollow granules during this early sex differentiation stage. These results provide novel information on a distinct stage of germ cell development and suggest different stage-specific roles for germ granules (Figure 3).

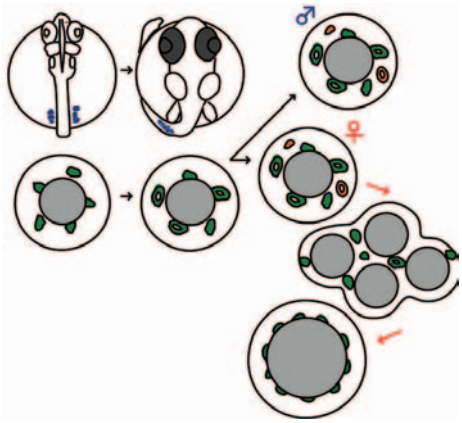

Figure 3. Change of localization of germ granule components olvas, nanos and tudor.

Publication List

〔Original papers〕

- Aoki, Y., Nagao, I., Saito, D., Ebe, Y., Kinjo, M., and Tanaka, M. (2008). Temporal and spatial localization of three germline-specific proteins in medaka. Dev. Dyn. *273*, 800-807.
- Nagao, I., Aoki, Y., Tanaka, M., and Kinjo, M. (2008). Analysis of the molecular dynamics of medaka nuage proteins by fluorescence correlation spectroscopy and fluorescence recovery after photobleaching. FEBS J. *275*, 341–349.
- Nakabayashi, T., Nagao, I., Kinjo, M., Aoki, Y., Tanaka, M., and Ohta, N. (2008). Stress-induced environmental changes in a single cell as revealed by fluorescence lifetime imaging. Photonchem. Photobiol. Sci. *7*, 671-674.
- Nakamura, S., Aoki, Y., Saito, D., Kuroki, Y., Fujiyama, A., Naruse, K., and Tanaka, M. (2008). *Sox9b/sox9a2*-EGFP transgenic medaka reveals the morphological reorganization of the gonads and a common presursor of both the female and male supporting cells. Mol. Reprod. Dev. *75*, 472-476.
- Sasado, T., Yasuoka, A., Abe, K., Mitani, H., Furutani-Seiki, M., Tanaka, M., and Kondoh, H. (2008). Distinct contributions of CXCR4b and CXCR7/RDC1 receptor systems in regulation of PGC migration revealed by medaka mutants *kazura* and *yanagi*. Dev. Biol. *320*, 328-339.

〔Review articles〕

- Nakamura, S., Saito, D., and Tanaka, M. (2008). Generation of transgenic medaka using modified bacterial artificial chromosome. Dev. Growth Differ. *50*, 415-417.
- Tanaka, M., Saito, D., Morinaga, C., and Kurokawa, H. (2008). Cross talk between germ cells and gonadal somatic cells is critical for sex differentiation of the gonads in the teleost fish, medaka (*Oryzias latipes*). Dev. Growth Differ. *50*, 273-278.