## Laboratory of Molecular Genetics for Reproduction †

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### Laboratory scope

Reproduction is a universal and fundamental system for organisms to produce new generations. To accomplish this purpose, organisms have developed their own sexual strategies, which allow them to adapt to their environment, thereby progressing toward maximum efficiency of reproduction. During the embryo and larval terms, organisms develop many cell-lineages that have special and essential roles in each different process of reproduction. These lineages are mostly conserved among vertebrates.

Vertebrates, however, exhibit a variety of reproductive systems. The mechanisms of sex determination and sex differentiation are some of the components of the reproductive system which produce this variety. Actually, there are many modes of sex determination. Sex determination genes are different among vertebrates. Sex determination does not even have to be controlled genetically. This variety is allowed by the different employment and different emergence of the cell lineages during embryogenesis. Therefore, it is important to address the roles of each cell lineage for understanding the fundamental mechanism underlying a variety of reproductive systems. Currently, our lab focuses on the core mechanisms which are independent of sex determination genes and which produce and maintain the sex. The core mechanism can be referred to as cellular interaction between germ cells and surrounding somatic cells, wherein germ cells have the ability to feminize somatic cells while the surrounding somatic cells are predisposed to male development. These characters of each set of cells are totally independent of the sex determination gene on the medaka Y chromosome. We are addressing the details of this core mechanism by analyzing each cell lineage in the context of sex differentiation.

To accomplish the purpose of our study, we use medaka fish (*Oryzias latipes*). We have been generating transgenic medaka enabling us to analyze how different cell lineages are involved in the process of gonad formation and sex differentiation *in vivo*. Additionally, in order to identify the genes essential for reproduction, we carried out a mutational screening of medaka with defective phenotypes and disrupted several candidate genes. With these two unique analytical methods (visualizing cells, and mutants), we are attempting to unveil both the fundamental mechanisms and the specific mechanisms that produce a variety of reproductive systems.

†: This laboratory was closed on 31 May, 2016.

Through these analyses, we have been revealing the presence of germline stem cells in the ovary. This was the first proof of this system in vertebrates (Nakamura et al 2010 Science). The fluorescently labeled germline stem cells keep producing eggs with fluorescence during the entire period of medaka reproduction, which is a conclusive indication of the presence of germline stem cells. The tricks employed in this experiment are transgenic medaka that allow heat-inducible gene expression.

### I. Identification of a fate switch gene in germ cells (sex determination gene of germ cells)

In the core mechanism of sex, germ cells are responsible for feminization and somatic cells are for masculinization. An important thing here is that we can determine the sex intentionally, if the core mechanism is to be modified, without any effect from the sex determination gene. Then a big issue is how the sex of germ cells, in other words, the fate decision of germ cells to become sperm or eggs, is determined. Few people have addressed this issue in vertebrates.

It is generally accepted that germline stem cells are sexually indifferent or unfixed. And the appearance of germ cells remains the same during the process of both spermatogenesis and oogenesis until germ cells enter meiosis. This suggests that the sex (the fate decision to become eggs or sperm) might not be determined by that time.

Based on these observations, we revealed the genes which differentially express in germ cells just before meiosis. Among them, we successfully identified the gene that determines the sexual fate in germ cells. The gene is one of the genes encoding forkhead transcriptional factors, *foxl3*. The mutant analysis indicated that the factor functions in germ cells autonomously and represses entering spermatogenesis in germ cells. The mutant did not cause any defect in sex differentiation of somatic cells – The XX mutant, genetically female medaka, develops a normal ovary but germ cells develop into sperm in the mutant ovary during young adult period. Artificial insemination with sperm isolated from the mutant ovary and eggs from the wildtype ovary produces fertile eggs, demonstrating that the sperm in the mutant ovary are functional.

### II. Structural origins of ovary and testis.

Structural analysis of the mutant ovary leads to one interesting view on the origins of ovary and testis.

The view suggests that indifferent gonads have a common unit for both germinal cradles and lobules. Germinal cradles identify niche regions of germline stem cells in the ovary, and lobules in the testis harbor germline stem cells at their distal ends. Both structures develop from the common unit composed of supporting cells that surround germ cells, which is underlaid by the basement membrane. A mature ovary is composed of both germinal epithelium where germinal cradles (germline niche) are present and the stromal compartment where vitellogenic and/or mature oocytes are present.

Interestingly, the ovary in XX (genetically female) *foxl3* mutant develops a huge expansion of germinal epithelium and sperm is formed in the germinal epithelium. Although the mutant ovary possesses the stromal compartment,

sperm are never observed there. In the expanded area of the germinal epithelium, we can observe common units of germline stem cell-niche, supporting cells with basement membrane. These suggest that the expanded areas within the germinal epithelium are equivalent to testicular lobules.

All the observations mentioned above collectively suggest that testis in the wildtype male can be viewed as the structure organized and developed within the germinal epithelium. In other words it may be possible to assert that mature testis are developed from an origin (a prototype) of germinal epithelium. This is in sharp contrast with the ovary which is composed of germinal epithelium and stromal compartment. Mature eggs are developed in the stromal compartment and the germinal epithelium serves to provide an early stage of diplotene oocytes.

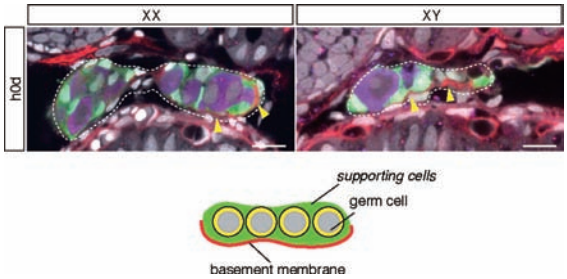


Figure 1. A common unit of ovary and testis. Supporting cells (green) that surround germ cells (yellow) and basement membrane (red) can be viewed as a common unit (below illustration) which develops into the niche structure of ovary (germinal cradles) or of testis (lobules). The unit is observed in both XX and XY gonads (upper images) where yellow arrow heads indicate deposition of basement membrane (red) and supporting cells are stained green. purple: germ cells, white: nuclea

## III. Environment during the larval period may affect sex of medaka.

As stated in the beginning, the sex of medaka is genetically determined. The sex determination gene on the Y chromosome determines the male (testicular) fate. However, our result suggests that the sex could be affected by the status of nutrients during the larval stage. If medaka larva is put under food-restricted conditions, the sex ratio of medaka is male-biased. Examination of the developing gonad indicates that the number of typeI germ cells (stem like germ cells) under the restricted conditions are reduced when compared to that under normal conditions. This result may suggest that the sex reversal effect by food restriction may be through germ cell number, which we had reported in previous papers.

### Publication List:

〔Review articles〕


- Tanaka, M. (2016). Germline stem cells are critical for sexual fate decision of germ cells. BioEssays *38*, 1227-1233.
- Nishimura, T., Nakamura, S., and Tanaka, M. (2016). A structurally and functionally common unit in testes and ovaries of medaka (*Oryzias latipes*), a teleost fish. Sex. Dev. *10*, 159-165.
- Nishimura, T., and Tanaka, M. (2016). The mechanism of germline sex determination in vertebrates. Biol. Reprod. *95*, 1-6.