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 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 defective gonads and are performing a positional cloning. With these two unique analytical methods (visualizing cells and mutants), we are attempting to unveil the fundamental mechanisms of sex differentiation and plasticity common to many organisms.

Figure 1. Larva of Various transgenic medaka.

I. Homeostasis in continous gamete production

To produce gametes throughout the reproductive period, there must be some mechanism of homeostasis ensuring the continuum of gamete production in the gonads. In mammalian testis, germline stem cells have been identified as a critical component for this homeostasis. On the other hand in female mammals, all the germ cells differentiate into oocytes during ovarian development in the fetus and the pool of oocytes is the sole sourse for egg production during the reproductive period in adult ovaries. This view of homeostasis has been widely accepted in mammals and is described in many textbooks.

However, many vertebrates with high fecundity produce an enormous number of eggs. In some vertebrates, the egg number reaches 10 to the order of 8 at a single spawning and they spawn eggs several times during their life cycles. It is very unlikely that the conventional theory of an oocyte pool developed during a fetal period can explain the large number of mature eggs.

Unlike mammalian ovaries, ovaries with high fecundity possess germ cells that undergo mitotic division. These immature germ cells are very likely to be the source of egg production, but the homeostasic mechanism that achieves this continuous and prolific egg production remains unknown.

II. *Sox9b*-expressing cells form ovarian cords in the germinal epithelium of ovary.

Sox9 is essential for initiation of testis formation and is under transcriptional regulation of mammalian testis determing gene, *Sry*, on the Y chromosome. *Sox9* is expressed not in the ovary but in Seroli cells of the testis that harbor germline stem cells.

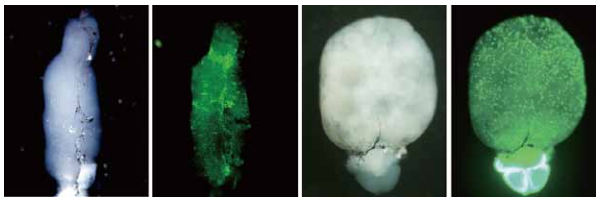

Figure 2. *sox9b* expression (green) in medaka adult testis (left two panels) and ovary (right two panels)

Previously we established transgenic medaka that recapitulate endogenous *sox9b* (medaka orthologue of mammalian *sox9*) expression by fluorescence. Interestingly, unlike mammals, medaka *sox9b* is expressed in both testis and ovary (Figure 2). The cells expressing *sox9b* reside in the ovarian structure called the germinal epithelium, which separates the stromal compartment, where oocyte growth occurs, from the ovarian cavity, into which mature oocytes are ovulated. *Sox9b*-expressing cells form a network by thin cellular processes. Since there have been no reports on this structure, the networks composed of *sox9b*-expressing cells were named “ovarian cords” (Figure 3).

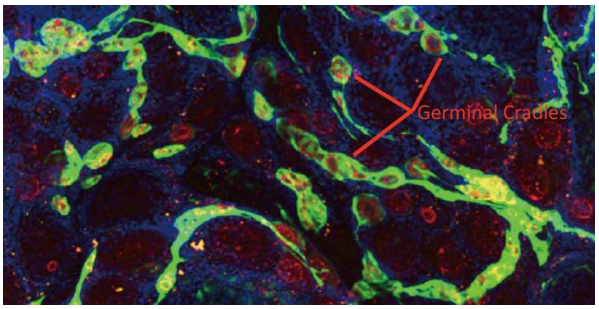

Figure 3. *Sox9b*-expressing cells (green) form a network structure called “ovarian cords” on the surface of adult medaka ovary. The germ cells (red) are endosed with the *sox9b*-expressing cells.

III. Germinal cradles in the ovarian cords contain germ cells at an early stage of oogensis.

Very intriguingly, all the germ cells at an early stage of oogenesis are colonized in the ovarian cords. Therefore we designated the colonized regions within the cords as the “germinal cradle” (Figure 4).

There are three types of germ cells found in the germinal cradles, germ cells that are isolated from the others by *sox9b*-expressing cells (Gs type), cyst-forming germ cells (Gcys type) and an early diplotene stage of oocytes (Gdip). Further

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 (*).

analysis with BrdU labeling experiments identified two populations of Gs type germ cells, rapidly and slowly dividing germ cells. Gcys germ cells divide synchronously three to five times to produce 8 to 32 clustered germ cells and then enter meiosis. Experiments with selective elimination of Gcys germ cells have suggested that Gcys cells were recovered from Gs cells. All the results have suggested that Gdip oocytes in the germinal cradles originate from slow-dividing Gs germ cells through Gcys germ cells and Gs germ cells may contain germline stem cells.

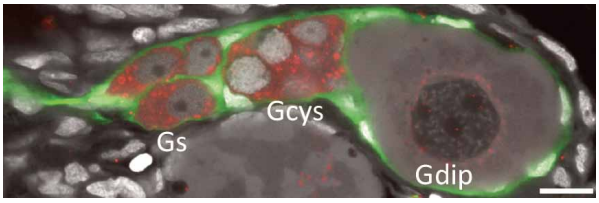

Figure 4. All the germ cells at early stages of oogenesis are clustered within ovarian cords. These colonized regions are called "germinal cradle" and contain germline stem cells.

IV. Germinal cradles in the ovarian cords harbor germline stem cells.

In order to prove that the Gs populations contain germline stem cells, clonal analysis was done. Previously we have shown that *nos2* is expressed in oogonial cells (Aoki et al, 2009 *Zool. Sci.*). Therefore we suspected that *nos2*-expressing germ cells correspond to a Gs type of germ cells and contain germline stem cells. The transgenic medaka was established in which GFP transcripts with *olvas*3'UTR can be driven by *nos2*-promoter upon heat treatment. Since *olvas*3'UTR functions to stabilize and enhance the translation in a *cis*-acting manner in all types of germ cells, this system allows us to keep track of progenitor cells from *nos2*-expressing cells.

Right after heat treatment, Gs germ cells were marked by fluorescence. But, as time passed by, all types of germ cells in the germinal cradles were labeled with fluorescence (Figure 5). Furthermore, we could obtain eggs and embryos from the marked *nos2*-expressing cells for three months.

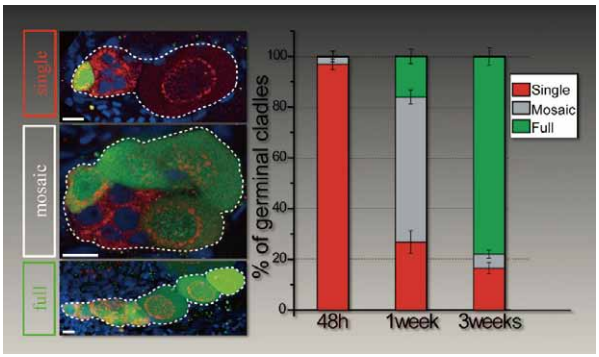

Figure 5. Clonal analysis of *nos2*-expressing cells in germinal cradles, proving the presence of germline stem cells.

These results clearly indicate that *nos2*-expressing germ cells in the germinal cradles contain germline stem cells that keep supplying eggs. This is the first demonstration of neo-oogenesis in adult ovary of vertebrates (Figure 6) (Nakamura et al., 2010 Science).

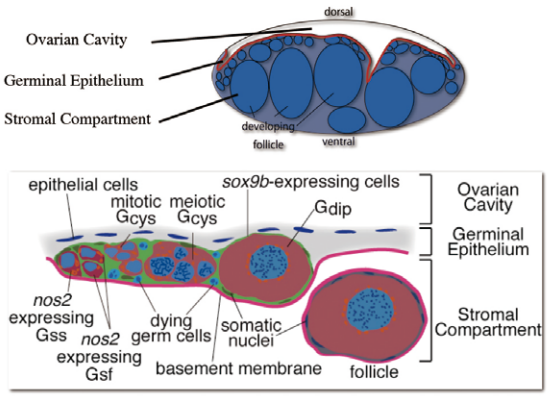

Figure 6. Upper figure indicates location of germinal epithelium in adult medaka ovary. Lower panel shows schematic representation of early oogenesis. Early oogenesis proceeds in the germinal cradles within the germinal epithelium.

Publication List

〔Original papers〕

- Herpin, A., Braasch, I., Kraeusslling, M., Schmidt, C., Thoma, E.C., Nakamura, S., Tanaka, M., and Schartl, M. (2010). Transcriptional rewiring of the sex determining *dmrt1* gene duplicate by transposable elements. PLoS Genetics *6*, e1000844.
- Ishikawa, T., Kamei, Y., Otozai, S., Kim, J., Sato, A., Kuwahara, Y., Tanaka, M., Deguchi, T., Inohara, H., Tsujimura, T., and Todo, T. (2010). High-resolution melting curve analysis for rapid detection of mutations in a Medaka TILLING library. BMC Mol. Biol. *11*, 70.
- Nakamura, S., Kobayashi, K., Nishimura, T., Higashijima, S., and Tanaka, M. (2010). Identification of germline stem cells in the ovary of teleost medaka. Science *328*, 1561-1563.

〔Review article〕

- Sasado, T., Tanaka, M., Kobayashi, K., Sato, T., Sakaizumi, M., and Naruse, K. (2010). The National BioResource Project Medaka (NBRP Medaka): an integrated bioresource for biological and biomedical sciences. Experimental Animals *59*, 13-24.