Laboratory of Molecular Genetics for Reproduction

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Laboratory scope

Reproduction is a universal and fundamental system for organisms to produce generations. To accomplish this purpose efficiently, organisms develop sexual 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 conserved among vertebrates.

Vertebrates, however, exhibit a variety of reproductive systems. 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 reproduction and a variety of reproductive systems. Currently, our lab focuses on the mechanisms of sex differentiation. The many modes of sex differentiation are a main component that contributes to variety, and we are addressing the role of each cell lineage in the context of sex differentiation.

We use medaka fish (*Oryzias latipes*) and 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.

Through these analyses, we have been revealing the importance of germ cells not only in sex differentiation but also in reproduction. Germ cells have been generally considered to be a permissive cell type that are completely regulated by the surrounding somatic cells. But we have found that the germ cells are not specialized for gametogenesis but are more self-determinant cells in reproduction.

I. Developmental origin of primordial germ cells

Development of germ cells essentially rely on the presence of a cytoplasmic structure, called germplasm or alternatively nuage. The lack of germplasm results in defective or loss of the germ cells. Two separate systems controlling the development of germ cells are known for vertebrates. Some vertebrate species possess a maternal origin of germplasm and the germplasm localized in a specific cell type is essential for germ cell development. In these species, therefore, cells that develop into germ cells can be identified by the presence of germplasm. On the other hand in the second method, like in mammals, germ cells are induced at the gastrulation stage. The germplasem-equivalent structures in these cells are also formed at a later stage.

Our previous studies indicated that, in medaka, germ cells are established as primordial germ cells (PGCs) during gastrulation. However, it is not known how a small population of blastodermal cells are specified as cells to produce PGCs, or if germ cells are induced like those seen in mammals. We have cloned a cDNA encoding Bucky ball, a component of germplasm. Since we have found *bucky ball* expressed in fertilized eggs, CFP-fused Bucky balls allow us to keep track of cells that could generate PGCs during early embryogenesis by confocal microscopy.

The RNA encoding *cfp*-fused *bucky ball* was injected into fertilized eggs. We found that the protein is readily translated and was observed as early as the onset of the first division. The CFP-fused Bucky ball protein is localized as several particles uniformly in the blastodisc. Very interestingly, the particles seem to be anchored at yolk layers until the late morula stage and seem independent of cell division in the blastodisc. The inhibition of the cleavage plane by nocodazol did not change any morphology or distribution of the particles, supporting the independence of the cleavage plane.

Nanos3 protein is known to be expressed in the established PGCs. Co-injection of *dsred-nanos*3'UTR RNA and *cfp*-fused *buckey ball* RNA indicates that DsRed-derived fluorescence begins to be detected in the cells that retain large CFP-fused Bucky ball particles. This clearly indicates that the cells retaining the Bucky ball particles are the presumptive germ cells that have the potential to be established as primordial germ cells.

Interestingly, at one or two stages, numerous Bucky ball granules are present with a range of different sizes, but, as

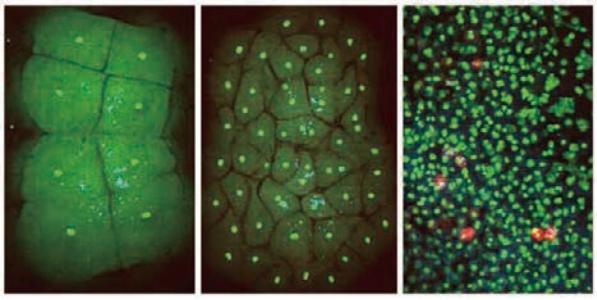

Figure 1. The localization of CFP-fused Bucky ball protein (blue) reveals presumptive primordial germ cells after fertilization. Green dots indicate histone3 signals in the nuclei. The cells expressing *nanos3* are established as primordial germ cells at the late morula stage, which are clearly recognized as the cells possessing large blue Bucky ball particles. Red cells are *nanos3*-expressing cells. The pictures are time-lapse shots of a live imaging movie. Left: 8 cell stage, Middle: 64 cell stage and Right: late morula stage.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2014. The former title is indicated by an asterisk (*).

development proceeds, small particles are getting eliminated. As a result, at the later morula stage, approximately 20-30 cells possess granules larger than 25 μm^3 and become PGCs. As the PGCs are established, the translation of nanos3 and vasa genes is activated. These translated proteins begin to be detected on the granules of Bucky balls.

II. Sexually different characteristics of primordial germ cells

Since 2013, we have been characterizing female and male primordial germ cells and have identified genes showing sexually different expression in the PGCs. Last year we reported that the sexually different expression of one of the identified genes, *sdgc*, is due to the presence of Y chromosomes but is not dependent on the medaka sex determination gene, *DMY*/*Dmrt1bY*.

This year, we continued to analyze the function of this gene and found that the gene regulates proliferation of primordial germ cells. Primordial germ cells isolated from male embryos proliferate more than those from female embryos in the culture dish. Loss of gene function by injection of morpholino results in a reduced proliferation rate in males while injection of *sdgc* RNA shows increasing activity of proliferation *in vitro*. These results suggest that the expression of *sdgc* confers primordial germ cells with the potential to regulate proliferation.

Furthermore, we have mapped *sdgc* on the medaka genome and found that *sdgc* is located very close to the *DMY*/*Dmrt1bY* locus. The *DMY*/*Dmrt1bY* locus is known to be the only region that is specific to Y chromosomes and where recombination is repressed. Consistent with this, we could find the sex-specific SNPs in the promoter and the intron regions of *sdgc*. *Sdgc* might represent the evolutional way of the Y chromosome differentiating into a more specialized chromosome.

Collectively, a series of analyses of *sdgc* indicate that cells have the ability to express sexually different characteristics autonomously, which is independent of the expression of sex determination gene.

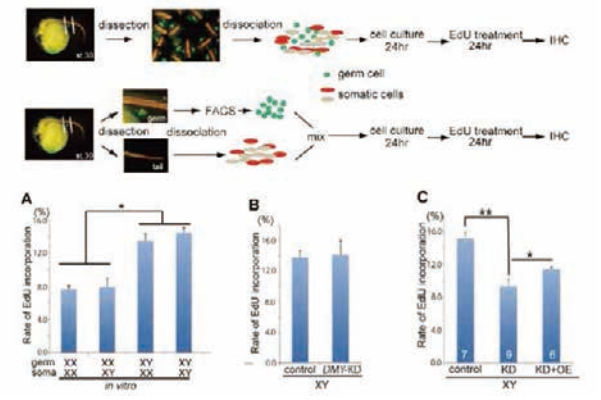

Figure 2. Sexually different behavior of primordial germ cells. Upper schema shows the way of primordial germ cell isolation and culture with somatic cells. A: Germ cell behavior (incorporation of EdU) is not affected by the sex of somatic cells but is determined cell-autonomously. B: The germ cell behavior is not dependent on the expression of sex determination gene, *DMY*/*Dmrt1bY*. C: The germ cell behavior (activity of proliferation) is reduced by knockdown of *sdgc*, which is recovered by overexpression of *sdgc*.

III. Sexually different development of steroidenic cells

Estrogen expression is important for feminization of the gonad, especially forming the ovarian cavity. Aromatase is a critical enzyme for producing estrogen from its precursor steroid, testosterone, and is encoded by the *cyp19a1* gene. Unlike mammals, during development the first emerging cells that express *cyp19a1* are the precursors of theca cells (in mammals, granulosa cells first express *cyp19a1*).

We have examined the development of steroidenic cells that produce testosterone and found that *ftz-f1* cells may be the precursor of testosterone-producing cells. Currently, two types of *ftz-f1* cells are observed in the developing gonads and precise lineage analysis is under investigation using time-lapse movies with the primary culture system.

Publication List

〔Original papers〕

- Nishimura, T., Herpin, A., Kimura, T., Hara, I., Kawasaki, T., Nakamura, S., Yamamoto, Y., Saito, T.L., Yoshimura, J., Morishita, S., Tsukahara, T., Kobayashi, S., Naruse, K., Shigenobu, S., Sakai, N., Schartl, M., and Tanaka, M. (2014). Analysis of a novel gene, *Sdgc*, reveals sex chromosome-dependent differences of medaka germ cells prior to gonad formation. Development *141*, 3363-3369.
- Okuyama, T., Yokoi, S., Abe, H., Isoe, Y., Suehiro, Y., Imada, H., Tanaka, M., Kawasaki, T., Yuba, S., Taniguchi, Y., Kamei, Y., Okubo, K., Shimada, A., Naruse, K., Takeda, H., Oka, Y., Kubo, T., and Takeuchi, H. (2014). A neural mechanism underlying mating preferences for familiar individuals in medaka fish. Science *343*, 91-94.

〔Review articles〕

- Tanaka, M. (2013). Vertebrate female germline—the acquisition of femaleness. WIREs Dev. Biol. doi: 10.1002/wdev.131
- Nishimura, T., and Tanaka, M. (2014). Gonadal development in fish. Sex. Dev. *8*, 252-261.
- Tanaka, M. (2014). Molecular and cellular bases of sexual flexibility in vertebrates. In 'New Principles in Developmental Processes (eds: Kondoh, H., and Kuroiwa, A.)' Springer Japan, pp. 265-278.