LABORATORY OF BIORESOURCES

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Medaka is a small egg-laying "secondary" fresh water fish found in brooks and rice paddies in Eastern Asia. This species has a long history as an experimental animal, especially in Japan. Our laboratory has conducted studies on evolution of the sex determination system using medaka and relatives, identification of the causal gene of mutants for PGC migration and pigment cell development, and the gonadal development of medaka. In addition to these activities, our laboratory is stepping forward to lead the National BioResource Project Medaka (NBRP Medaka).

I. Evolution of the sex chromosome and sex determination genes in *Oryzias* fish

Recent studies have demonstrated that *Oryzias* species have different genetic sex-determination systems (XX/XY and ZZ/ZW) (Figure 1). Furthermore, the sex chromosomes differ in their origin and degree of differentiation. These findings suggest the repeated creation of new sex chromosomes from autosomes during evolution of *Oryzias* fishes, possibly in association with the formation of new sex-determining genes. We are now trying to positionally clone the novel sex-determining genes in these species.

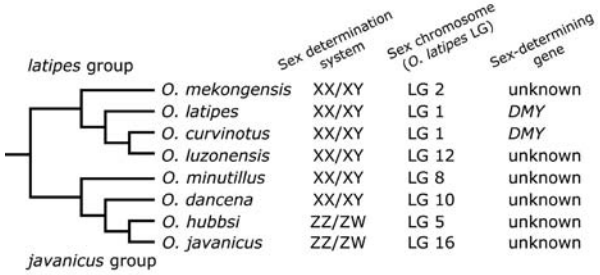

Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

Identification of these genes would provide a clue to understand the evolutionary process underlying frequent turnover of the sex determination mechanisms.

II. Genetic dissection of migration of primordial germ cells in medaka

Germ cells are responsible for the sustainability of life over generations in many multicellular animal species. To elucidate the mechanisms underlying the development of primordial germ cells, we identified multiple mutations affecting the migration and development of the primordial germ cells in medaka in a prior large-scale mutagenesis screening project, and have analyzed a set of them to date. We focused on three mutants that have defects in primordial germ cell migration, *kamigamo*, *shimogamo,* and *naruto* that were isolated in the screening project. Positional cloning and analysis of the genes carrying the mutations are now in progress. In addition, two mutations, *kamigamo* and *shimogamo*, cause cystic pronephric ducts simultaneously with abnormal positioning of the primordial germ cells. Therefore, the analysis of these mutations will be important in giving basal knowledge underlying the mechanisms of human cystic kidney diseases.

III. The function of estrogen in the medaka ovary

Estrogen has been generally considered to play a critical role in the ovarian differentiation of teleost fish by Yamamoto's model. In medaka, estrogen treatment has induced functional male-to-female sex reversal. To clarify the function of estrogen during ovarian development, we examined the role of ovarian aromatase (*arom*), which is responsible for catalyzing the conversion of testosterone to estrogen. We isolated two tilling mutant strains of *arom*. In these tiling mutants, one amino acid in aromatase ORF altered the STOP codon. In the tilling mutant of *arom*, the ovaries seemed to develop normally. However, in adult fish, yolk accumulation and formation of ovarian cavity were not observed. In some ovaries, spermatogenesis was observed. These results suggest that estrogen is not involved in early ovarian differentiation but has a critical role in maintenance of ovarian differentiation.

IV. Positional cloning of pigment cell mutants in medaka

All kinds of pigment cells are derived from neural crest cells. How each type of pigment cells differentiate and what differences are producing various pigment cell types is a very interesting question. Medaka have four types of pigment cell (melanophore, leucophore, xanthophore and iridocyte). The leucophore is unique because only some species have it. To elucidate how leucophore differentiate from neural crest cells and why it exists only in some fishes, we have successfully identified the causal gene of leucophore mutants (luecophore free (*lf*) and leucophre free 2 (*lf-2*)). We identified *slc2a15b* as the causal gene of the *lf* mutant. The *slc2a15b* expression exists but does not persist in the *lf* mutant. *slc2a15b* also exists in species without leucophore, but these use carotenoid as a pigment. Thus, *slc2a15b* may have an important role in

use of carotenoid as pigments. The *lf-2* phenotype was rescued by *pax7a*. *pax7a* is needed for differentiation of leucophore and xanthophore in medaka.

V. National BioResource Project Medaka (NBRP Medaka) (http://www.shigen.nig.ac.jp/medaka/)

In 2007, NIBB was selected as the core facility of NBRP Medaka. Our laboratory is taking an active part in this project. With the goal of facilitating and enhancing the use of medaka as a model organism, we provide, maintain and collect living resources such as standard strains, inbred strains, and mutants in addition to frozen resources such as EST/cDNA and BAC/ Fosmid clones and hatching enzymes, as well as integrated information on medaka (Figure 2). In 2011, we continued providing the TILLING screening system library to NBRP Medaka users for promoting the reverse genetic approach. NBRP Medaka aims to establish a first rate biological resource with the highest possible levels of accessibility and ease of use.

Figure 2. NBRP Medaka website

Publication List

〔Original papers〕

- Chakraborty, T., Shibata, Y., Zhou, L.Y., Katsu, Y., Iguchi, T., and Nagahama, Y. (2011). Differential expression of three estrogen receptor subtype mRNAs in gonads and liver from embryos to adults of the medaka, *Oryzias latipes*. Molecular and Cellular Endocrinology *333*, 47-54.
- Kai, W., Kikuchi, K., Tohari, S., Chew, A.K., Tay, A., Fujiwara, A., Hosoya, S., Suetake, H., Naruse, K., Brenner, S., *et al*. (2011). Integration of the Genetic Map and Genome Assembly of Fugu Facilitates Insights into Distinct Features of Genome Evolution in Teleosts and Mammals. Genome Biology and Evolution *3*, 424-442.
- Kato, M., Takehana, Y., Fukuda, Y., Naruse, K., Sakaizumi, M., and Hamaguchi, S. (2011). An autosomal locus controls sex reversal in interspecific XY hybrids of the medaka fishes. Heredity *107*, 523-529.
- Kobayashi, H., Iwamatsu, T., Shibata, Y., Ishihara, M., and Kobayashi, Y. (2011). Effects of co-administration of estrogen and androgen on induction of sex reversal in the medaka *Oryzias latipes*. Zoolog. Sci. *28*, 355-359.
- Koga, A., Sasaki, S., Naruse, K., Shimada, A., and Sakaizumi, M. (2011). Occurrence of a short variant of the Tol2 transposable element in natural populations of the medaka fish. Genetics Research *93*, 13-21.
- Okuyama, T., Suehiro, Y., Imada, H., Shimada, A., Naruse, K., Takeda, H., Kubo, T., and Takeuchi, H. (2011). Induction of c-fos transcription in the medaka brain (*Oryzias latipes*) in response to mating stimuli. Biochemical and Biophysical Research Communications *404*, 453-457.
- Paul-Prasanth, B., Shibata, Y., Horiguchi, R., and Nagahama, Y. (2011). Exposure to diethylstilbestrol during embryonic and larval stages of medaka fish (*Oryzias latipes*) leads to sex reversal in genetic males and reduced gonad weight in genetic females. Endocrinology *152*, 707-717.

〔Original paper (E-publication ahead of print)〕

- Takehana, Y., Naruse, K., Asada, Y., Matsuda, Y., Shin-I, T., Kohara, Y., Fujiyama, A., Hamaguchi, S., and Sakaizumi, M. Molecular cloning and characterization of the repetitive DNA sequences that comprise the constitutive heterochromatin of the W chromosomes of medaka fishes. Chromosome Res. 2011 Nov. 29.

〔Review articles〕

- Naruse, K. (2011). Genetics, Genomics, and Biological Resources in the Medaka, *Oryzias latipes*. In: Medaka, A Model for Organogesis, Human Diseases and Evolution. Naruse, K., Tanaka, M., and Takeda, H. eds. (Springer), pp. 19-37.
- Naruse, K. Tanaka, M., and Takada, H. (2011). Medaka, A Model for Organogesis, Human Diseases and Evolution Springer Tokyo.
- Shibata, N., Nakamoto, M., Shibata, Y., and Nagahama, Y. (2011) Endocrine Regulation of Oogenesisin the Medaka, *Oryzias latipes*. In: Medaka: A Model for Organogenesis, Human Disease, and Evolution. Naruse, K., Tanaka, M., and Takeda, H. eds. (Springer), pp. 267-283.
- Takehana, Y. (2011). Frequent turnover of sex chromosomes in the medaka fishes. In: Medaka, A Model for Organogesis, Human Diseases and Evolution. Naruse, K., Tanaka, M., and Takeda, H. eds. (Springer), pp. 229-240.