

## 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. Identification of these genes would provide a clue to understand the evolutionary process underlying frequent

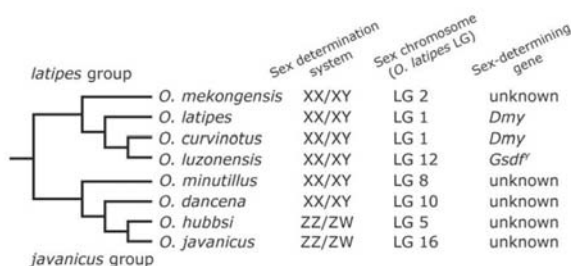


Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

turnover of 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

Estrogens have 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, which is responsible for catalyzing the conversion of testosterone to estrogen. We isolated two tilling mutant strains of ovarian aromatase. In these tilling mutants, one amino acid in ovarian aromatase ORF altered the STOP codon. Mutant females seemed to develop normal ovaries but yolk accumulation was not observed in the ovarian follicles and most of the ovarian follicles underwent atresia in the adult ovary. Even more surprisingly, spermatogenesis was observed within the mutant ovary. These results suggest that the hypothesis that endogenous estrogens drive ovarian differentiation needs to be modified to the hypothesis that estrogens are essential for the maintenance of ovarian differentiation.

### IV. Positional cloning of pigment cell mutants in medaka

Animal body colors are generated primarily by neural crest-derived pigment cells in the skin. Whereas mammals and birds have only melanocyte on the body surface, fish have a variety of pigment cell types, also referred to as chromatophores: melanophores, xanthophores, iridophores and so on. Compared with other fish species, medaka has a unique chromatophore type, leucophores. However, the genetic basis of chromatophore diversity has so far remained barely understood. Here, we found that three loci, leucophore free (*lf*), *lf-2* and white leucophore (*wl*), in medaka, which affect leucophore and xanthophore formation, encode *slc2a15b*, *pax7a* and *slc2a11b*, respectively. As *lf-2*, a loss-of-function mutant for *pax7a*, exhibits defects in formation of xanthophore and leucophore precursor cells, *pax7a* is critical

for fate specification of the xanthophore/leucophore lineage. This genetic evidence implies that leucophores develop in the same cell lineage as xanthophores, although it has been believed that leucophores are related to iridophores because they have the property of purine-dependent light reflection in common. Our identification of *slc2a15b* and *slc2a11b* as crucial for pigmentation of leucophores and xanthophores in medaka revealed that the existence of these two genes in the genome coincides with the trait of having xanthophores or their characteristic intracellular organelle in non-mammalian vertebrates. Considering that birds have yellow-pigmented irises with xanthophore-like intracellular organelles, our findings provide clues for uncovering evolutionary diverse mechanisms of pigment cell formation in animals.

## 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, BAC/ Fosmid clones and hatching enzymes, as well as integrated information on medaka (Figure 2). We have been providing BAC clones of medaka related species, a library screening system employing a 3D PCR strategy for evolutionary studies, and the TILLING screening system 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]

- Guan, G., Yan, Y., Chen, T., Yi, M., Ni, H., Naruse, K., Nagahama, Y., and Hong, Y. (2013). Nanos3 gene targeting in medaka ES cells. *Int. J. Biol. Sci.* 9, 444-454.
- Horiguchi, R., Nozu, R., Hirai, T., Kobayashi, Y., Nagahama, Y., and Nakamura, M. (2013). Characterization of gonadal soma-derived factor expression during sex change in the protogynous wrasse, *Halichoeres trimaculatus*. *Dev. Dyn.* 242, 388-399.
- Kawaguchi, M., Takahashi, H., Takehana, Y., Naruse, K., Nishida, M., and Yasumasu, S. (2013). Sub-functionalization of duplicated genes in the evolution of nine-spined stickleback hatching enzyme. *J. Exp. Zool. B Mol. Dev. Evol.* 320, 140-150.
- Nozu, R., Horiguchi, R., Murata, R., Kobayashi, Y., and Nakamura, M. (2013). Survival of ovarian somatic cells during sex change in the protogynous wrasse, *Halichoeres trimaculatus*. *Fish Physiol. Biochem.* 39, 47-51.
- Ohshima, A., Morimura, N., Matsumoto, C., Hiraga, A., Komine, R., Kimura, T., Naruse, K., and Fukumachi, S. (2013). Effects of body-color mutations on vitality: an attempt to establish easy-to-breed see-through medaka strains by outcrossing. *G3* 3, 1577-1585.
- Okuyama, T., Ise, Y., Hoki, M., Suehiro, Y., Yamagishi, G., Naruse, K., Kinoshita, M., Kamei, Y., Shimizu, A., Kubo, T., et al. (2013). Controlled Cre/loxP site-specific recombination in the developing brain in medaka fish, *Oryzias latipes*. *PLoS ONE* 8, e66597.
- Paul-Prasanth, B., Bhandari, R.K., Kobayashi, T., Horiguchi, R., Kobayashi, Y., Nakamoto, M., Shibata, Y., Sakai, F., Nakamura, M., and Nagahama, Y. (2013). Estrogen oversees the maintenance of the female genetic program in terminally differentiated gonochorists. *Sci. Rep.* 3, 2862.
- Uno, Y., Asada, Y., Nishida, C., Takehana, Y., Sakaizumi, M., and Matsuda, Y. (2013). Divergence of repetitive DNA sequences in the heterochromatin of medaka fishes: molecular cytogenetic characterization of constitutive heterochromatin in two medaka species: *Oryzias hubbsi* and *O. celebensis* (Adrianichthyidae, Belontiiformes). *Cytogenet. Genome Res.* 141, 212-226.

### [Original paper (E-publication ahead of print)]

- Zhang, X., Guan, G., Chen, J., Naruse, K., and Hong, Y. Parameters and efficiency of direct gene disruption by zinc finger nucleases in medaka embryos. *Marine Biotech.* 2013 Oct. 23.