

LABORATORY OF BIORESOURCES

Associate Professor
NARUSE, Kiyoshi

Assistant Professor	TAKEHANA, Yusuke
NIBB Research Fellow:	TAKEHANA, Yusuke*
Postdoctoral Fellow:	SASADO, Takao
	SHIBATA, Yasushi
	SHIBA, Keikun
	NAKAMOTO, Masatoshi
	OKUYAMA, Teruhiro
	KIMURA, Tetsuaki
	NOZU, Ryo
Research Fellow:	KANEKO, Hiroyo
	YOSHIMURA, Yuriko
Technical Assistant:	KOIKE, Yukari
	TORII, Naoko
	AJIOKA, Rie
	KOIKE, Chieko
	TESHIMA, Yuuko
	HARA, Ikuyo
	ISHIKAWA, Hiroe
	SHIBATA, Emiko
	TAKAKI, Chikako
	HAMATANI, Ayako
Secretary:	SUZUKI, Tokiko

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

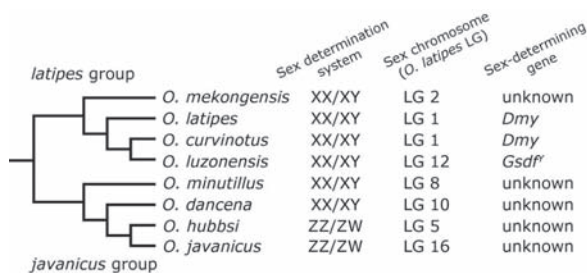


Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

the novel sex-determining genes in these species. Identification of these genes would provide a clue to understand the evolutionary process underlying frequent 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 tiling mutant strains of ovarian aromatase. In these tiling 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 undergo 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

All kinds of pigment cells are derived from neural crest cells. How each type of pigment cell differentiates and what differences are producing which of the various pigment cell types is a very interesting question. Medaka have four types of pigment cell (melanophore, leucophore, xanthophore and iridocyte). The leucophores are unique because only some species have them. To elucidate how leucophores differentiate from neural crest cells and why they exist only in some fishes, we have successfully identified the causal gene of leucophore mutants (leucophore free (*lf*) and leucophore 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 leucophores, 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.

IV. 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). We have been providing the TILLING screening system to NBRP Medaka users for promoting the reverse genetic approach since 2011. NBRP Medaka aims to establish a first rate biological resource with the highest possible levels of accessibility and ease of use.

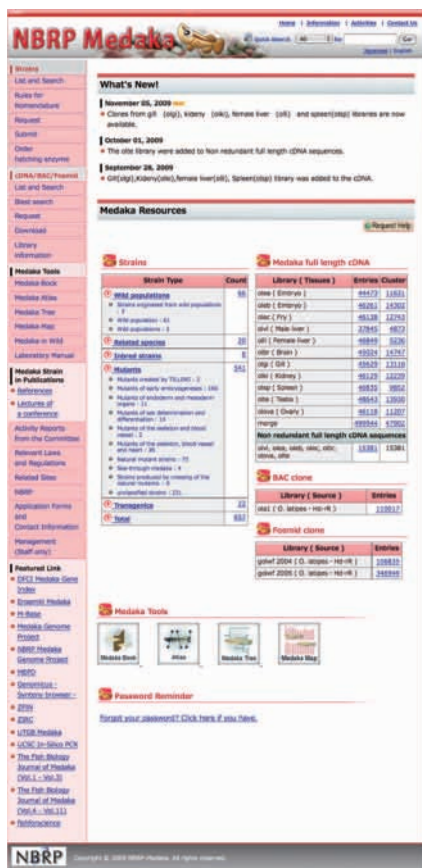


Figure 2. NBRP Medaka website

Publication List

[Original papers]

- Chen, J., Zhang, X., Wang, T., Li, Z., Guan, G., and Hong, Y. (2012). Efficient detection, quantification and enrichment of subtle allelic alterations. *DNA Research* 19, 423-433.
- Isoe, Y., Okuyama, T., Taniguchi, Y., Kubo, T., and Takeuchi, H. (2012). p53 Mutation suppresses adult neurogenesis in medaka fish (*Oryzias latipes*). *Biochem Biophys Res. Commun.* 423, 627-631.
- Kimura, T., and Naruse, K. (2012). Genetic analysis of vertebral regionalization and number in Medaka (*Oryzias latipes*) inbred lines. *G3* 2, 1317-1323.
- Li, J., Chen, W., Wang, D., Zhou, L., Sakai, F., Guan, G., and Nagahama, Y. (2012). GATA4 is involved in the gonadal development and maturation of the teleost fish tilapia, *Oreochromis niloticus*. *J. Reprod. Dev.* 58, 237-242.
- Moriyama, Y., Kawanishi, T., Nakamura, R., Tsukahara, T., Sumiyama, K., Suster, M.L., Kawakami, K., Toyoda, A., Fujiyama, A., Yasuoka, Y., Nagao, Y., Sawatari, E., Shimizu, A., Wakamatsu, Y., Hibi, M., Taira, M., Okabe, M., Naruse, K., Hashimoto, H., Shimada, A., and Takeda, H. (2012). The medaka *zic1/zic4* mutant provides molecular insights into teleost caudal fin evolution. *Curr. Biol.* 22, 601-607.
- Myosho T., Otake, H., Masuyama H., Matuda M., Kuroki Y., Fujiyama A., Naruse K., Hamaguchi, S., and Sakaizumi, M. (2012). Tracing the emergence of a novel sex-determining gene in medaka, *Oryzias latipes*. *Genetics* 191, 163-170.
- Myosho, T., Takehana, Y., Sato, T., Hamaguchi, S., and Sakaizumi, M. (2012). The origin of the large metacentric chromosome pair in Chinese medaka (*Oryzias sinensis*). *Ichthyol. Res.* 59, 384-388.
- Nakamoto, M., Fukasawa, M., Tanaka, S., Shimamori, K., Suzuki, A., Matsuda, M., Kobayashi, T., Nagahama, Y., and Shibata, N. (2012). Expression of 3 β -hydroxysteroid dehydrogenase (*hsd3b*), star and *ad4bp/sf-1* during gonadal development in medaka (*Oryzias latipes*). *Gen. Comp. Endocrinol.* 176, 222-230.
- Oshima Y., Sato H., Kajiura-Kobayashi H., Kimura T., Naruse K., and Nonaka, S. (2012). Light sheet-excited spontaneous Raman imaging of a living fish by optical sectioning in a wide field Raman microscope. *Optic Express.* 20, 16195-16204.
- Shibata, Y., Iwamatsu, T., Suzuki, N., Young, G., Naruse, K., Nagahama, Y., Yoshikuni, M. (2012). An oocyte-specific astacin family protease, alveolin, is released from cortical granules to trigger egg envelope hardening during fertilization in medaka (*Oryzias latipes*). *Develop. Biol.* 372, 239-248.
- Takehana, Y., Naruse, K., Asada, Y., Matsuda, Y., Shin-I, T., Kohara, Y., Fujiyama, A., Hamaguchi, S., and Sakaizumi, M. (2012). Molecular cloning and characterization of the repetitive DNA sequences that comprise the constitutive heterochromatin of the W chromosomes of medaka fishes. *Chromosome Res.* 20, 71-81.
- Zhao, H., Li, M., Purwanti, Y.L., Liu, R., Chen, T., Li, Z., Hong, N., Guan, G., Yin, A., Xiao, L., Ge, R., Song, J., and Hong, Y. (2012). *Mitf* is a transcriptional activator of medaka germ genes in culture. *Biochemie* 94, 759-767.

[Original papers (E-publication ahead of print)]

- Li, M., Guan, G., Hong, N., and Hong, Y. Multiple regulatory regions control the transcription of medaka germ gene *vasa*. *Biochimie* 2012 Dec 8.
- Zhao, H., Guan, G., Duan, J., Cheng, N., Wang, J., Matsuda, M., Paul-Prasanth, B., and Nagahama, Y. OI4E-T, a eukaryotic translation initiation factor 4E-binding protein of medaka fish (*Oryzias latipes*), can interact with *nanos3* and *vasa* *in vitro*. *J. Exp. Zool. B (Mol. Dev. Evol.)* 2012 Sep 5.

[Review article]

- Kimura, T., Kamei, Y., Takehana, Y., Sasado, T., and Naruse, K. (2012). Medaka genomes and the methods and resources for decoding genomic functions. In *Genome Mapping and Genomics in Laboratory Animals*, P. Denny and C. Kole, eds. (London Springer), pp159-182.