LABORATORY OF BIORESOURCES

Associate Professor
NARUSE, Kiyoshi

Postdoctoral Fellows:	*SASADO, Takao*
	TAKEHANA, Yusuke
NIBB Research Fellow:	*KIMURA, Tetsuaki*
Research Fellows:	*KANEKO, Hiroyo*
	YOSHIMURA, Yuriko
Technical Assistants:	*KOIKE, Yukari*
	TORII, Naoko
	AJIOKA, Rie
	KOIKE, Chieko
	HOSOMI, Azusa
	TESHIMA, Yuuko
	HARA, Ikuyo
Secretary:	*SUZUKI, Tokiko*

Teleosts comprise about half of all vertebrate species and have adapted to a variety of environments, including sea-water, fresh water, the bottom of deep seas, small creeks and paddy fields. Analysis of their genome structure is important in order to understand the adaptation and diversification of this interesting group. 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 a comparative genomic analysis focusing mainly on fish chromosomes and gene evolution using medaka and other fishes, and identification of the causal gene of mutants for PGC migration. In addition to these activities, our laboratory is stepping forward to lead the National BioResource Project Medaka (NBRP Medaka).

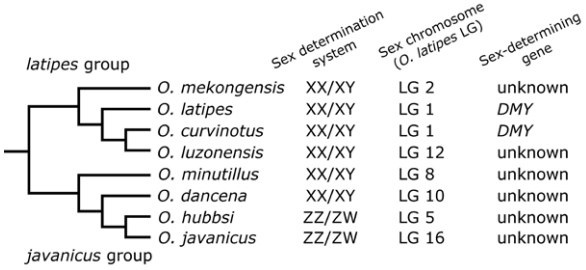

Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

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

The sex-determining gene *DMY* was identified on the Y chromosome of medaka, *Oryzias latipes*. However, this gene is absent in most *Oryzias* fishes, suggesting that closely related species have different sex-determining genes. We have recently demonstrated that, in the *javanicus* species group, *O. dancena* and *O. minutillus* have an XX/XY sex determination system, while *O. hubbsi* and *O. javanicus* have a ZZ/ZW system (Figure 1). Linkage analysis and FISH analysis showed that the sex chromosomes in these species were not homologous, suggesting independent origins of these sex chromosomes. Furthermore, *O. javanicus* and *O. hubbsi* have morphologically heteromorphic ZW sex chromosomes, in which the W chromosome has DAPI-positive heterochromatin. These findings suggest the repeated evolution of new sex chromosomes from autosomes in *Oryzias*, probably through the emergence of a new sex-determining gene.

II. Genetic dissection of migration of primordial germ cells in the 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 past 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. M-marker 2009, a new marker set for medaka mutant chromosome assign.

When the causal genes of mutants are cloned using candidate gene approaches or by chromosomal walking the first step involves assignment of the mutant to a specific chromosome. Bulked segregation analysis has been the most frequently used and effective method for assigning mutant loci to specific chromosomes. The bulk segregation analysis measures allele frequencies in pools of segregates that have been sorted according to phenotype. F2 wild type pool derived from two different strains has the same allele frequency throughout genome. However, because the mutant pool has only one allele in causal mutation there is a DNA marker which is derived solely from the mutant strain. Then we can assign the mutant phenotype to a specific chromosome locus by comparison of allele frequency of the pools. In medaka, the M-marker 2009 primer set, which consists of 48 PCR length polymorphism (PLP) markers (two markers per chromosome) have been used to map mutants using this method. M-marker 2009 markers were designed to amplify genomic regions containing insertion/deletion (in-del) polymorphisms between the Southern and Northern Japanese medaka strains that are most frequently used for position-based cloning of causal genes in mutants.

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

4-1 Establishment of core facility of NBRP 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). NBRP Medaka aims to establish a first rate biological resource with the highest possible levels of accessibility and ease of use.

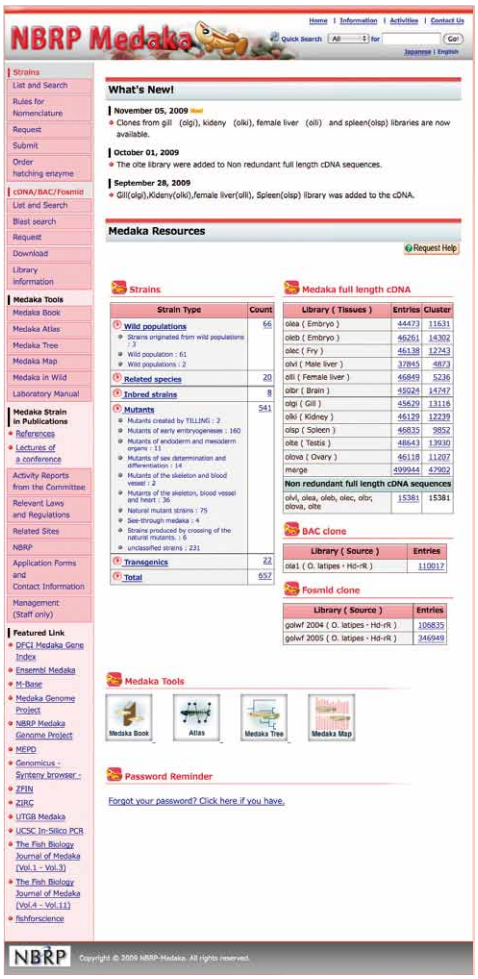

Figure 2. NBRP Medaka website

4-2 Establishment of polymorphism information of medaka inbred strains

One of the prominent characteristics of Medaka BioResources is the availability of inbred strains. These include several strain-specific characteristics such as vent of vertebrae during aging, acceleration of aging, shortened breeding duration, sensing of gravity, and maturation size etc. To analyze these strain-specific characteristics, the genome sequence data of each inbred line is crucial. The National Institute for Basic Biology and the National Institute of Genetics collaboratively conduct a genome sequencing project with a second generation sequencer for five representative inbred strains (Hd-rR-II1, HNI-II, Kaga, Nilan and HSOK) derived from Southern Japanese, Northern Japanese, China-West Korean and East Korean populations respectively. This data promotes quantitative trait loci (QTL) analysis using the inbred strains and facilitate the development of human disease models using the medaka system. In addition, the Ilumina GA sequence data from the Hd-rR-II1 strain enhance the reference genome sequence of medaka. Overall, this program brings medaka forward to a new level as a model system.

Publication List

〔Original papers〕

- Kato, M., Takehana, Y., Sakaizumi, M., and Hamaguchi, S. (2010). A sex-determining region on the Y chromosome controls the sex-reversal ratio in interspecific hybrids between *Oryzias curvinotus* females and *Oryzias latipes* males. Heredity *104*, 191-195.
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- Kuroyanagi, Y., Okuyama, T., Suehiro, Y., Imada, H., Shimada, A., Naruse, K., Takeda, H., Kubo, T., and Takeuchi, H. (2010). Proliferation zones in adult medaka (*Oryzias latipes*) brain. Brain Res. *1323*, 33-40.
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- Tani, S., Kusakabe, R., Naruse, K., Sakamoto, H., and Inoue, K. (2010). Genomic organization and embryonic expression of miR-430 in medaka (*Oryzias latipes*): insights into the post-transcriptional gene regulation in early development. Gene *449*, 41-49.
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〔Original papers (E-publication ahead of print)〕

- Koga, A., Sasaki, S., Naruse, K., Shimada, A., and Sakaizumi, M. (2010). Occurrence of a short variant of the Tol2 Transposable element in natural populations of the medaka fish. Genet. Research 2010 Dec 7.
- Okuyama, T., Suehiro, Y., Imada, H., Shimada, A., Naruse, K., Takeda, H., Kubo, T., and Takeuchi, H. (2010). Induction of c-fos transcription in the medaka brain (*Oryzias latipes*) in response to mating stimuli. Biochem. Biophys. Res. Commun. 2010 Dec 5.

〔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.