LABORATORY OF BIORESOURCES

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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 in 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).

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

The sex-determining gene *DMY* was identified on the Y chromosome in the 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.

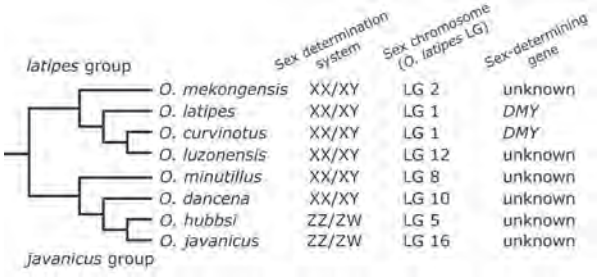

Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

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

The migratory pathways of PGCs to the gonad vary depending on the vertebrate species, yet the underlying regulatory mechanisms guiding PGCs are believed to be common between species. In the medaka embryo, PGC migration follows two major steps before colonizing in gonadal areas: (1) bilateral lineup in the trunk, and (2) posterior drift. *kazura* (*kaz*) and *yanagi* (*yan*) mutants of medaka isolated in our mutagenesis-screening were defective in the first and second steps, respectively. Positional cloning identified phenotype responsible mutations in the chemokine receptor genes *cxcr4b* and *cxcr7*, respectively. Although belonging to the same chemokine receptor families, involvement of these genes in the regulation of PGC migration was clearly distinct. *cxcr4b* is expressed in the PGCs themselves, suggesting a cell-autonomous function. In contrast, *cxcr7* is not expressed in the PGCs but in the mesoderm-derived tissues connected to the route of PGC migration, the ventral part of the somites in the area always immediately anterior to the PGCs drifting along the bilateral routes toward the gonad, and the pronephric ducts (Sasado et al., 2008). Further analysis of the mutants is underway to reveal the function of the two chemokine-systems in the regulation of PGC migration. *kamigamo* (*kmg*) and *shimogamo* (*smg*) are both recessive lethal mutations showing PGC distribution defects similar to that of *yan* but in different complementation groups (Sasado et al., 2004). Positional cloning of the responsible genes of the mutations is now in progress.

III. The development of insertional mutagenesis system with Tol2 transposon

Mutagenesis screens by N-ethyl-N-nitrosourea have been performed in medaka. However, the cloning of chemically mutated genes is still laborious even after completion of medaka genome sequencing. In order to rapidly identify a causal mutation of phenotype, we undertake insertional mutagenesis in medaka using a transposable element, Tol2.

Tol2 is a transposable element of the hAT transposable element family, residing in the genome of the medaka. The Hd-rR, an inbred strain sequenced in the genome project, has 14 integration sites in its genome. These sites are stably inherited in the Hd-rR.

We demonstrated that Tol2 can be excised in the Hd-rR genome when transposase mRNA, synthesized in vitro, is injected. Multiple different excisions occurred in founder fish. These mutations were highly heritable, however due to the mosaic like nature of the germ cells progeny showed various new mutations. Our results show that Tol2 is useful as a mutagen in medaka.

IV. National BioResource Project Medaka (NBRP Medaka)

4-1 Full length cDNA sequencing project

To establish the full length cDNA resources of medaka we made 11 full length cDNA libraries (developmental stage 22, 35, 40, ovary, testes, brain, male liver, female liver, gill, kidny and spleen) and determined the sequences of both ends of 250,000 clones in collaboration with the National Institute of Genetics' Kohara and Fujiyama labs. Now 499,944 sequences are availabe. After mass alignment of all sequences, we found 21,588 independent sequences at the 3' ends. All of the data was deposited in the DDBJ and is accessible from the National BioResource Project Medaka website (http://www.shigen.nig.ac.jp/medaka/).

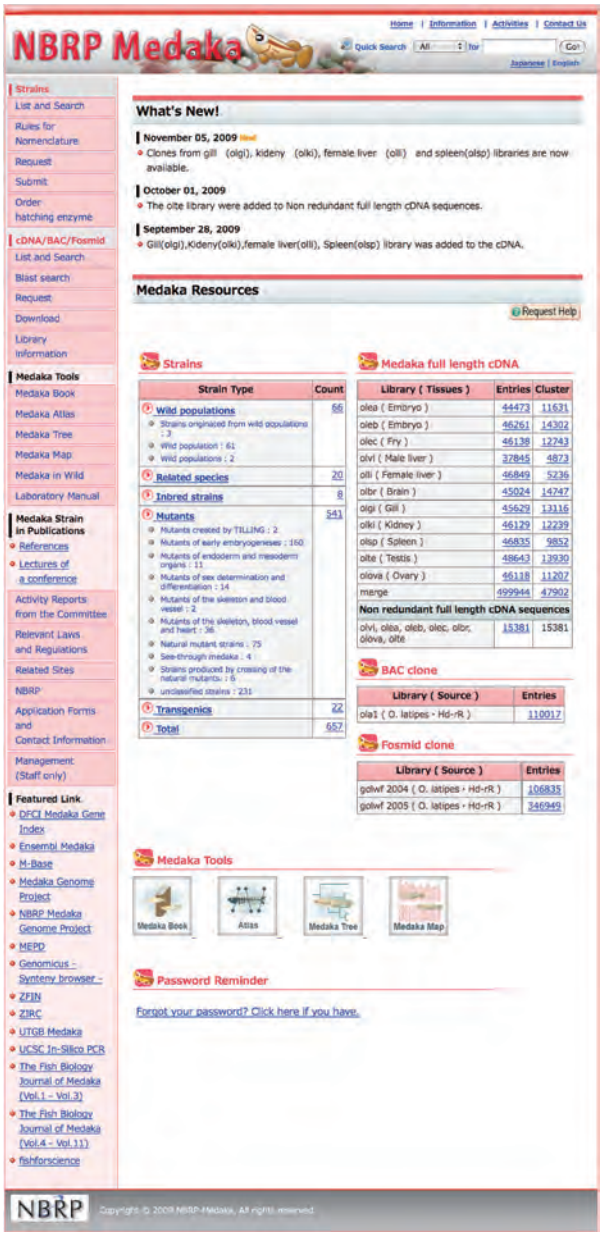

Figure 2. NBRP Medaka website

4-2 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.

Publication List

〔Original papers〕

- Abe, K., Klaften, M., Narita, A., Kimura, T., Imai, K., Kimura, M., Rubio-Aliaga, I., Wagner, S., Jakob, T., and Hrabé de Angelis, M. (2009). Genome-wide search for genes that modulate inflammatory arthritis caused by Ali18 mutation in mice. Mamm Genome. *20*,152-161.
- Hashimoto, H., Miyamoto, R., Watanabe, N., Shiba, D., Ozato, K., et al. (2009). Polycystic kidney disease in the medaka (*Oryzias latipes*) pc mutant caused by a mutation in the Gli-similar3 (glis3) gene. PLoS ONE *4*, e6299.

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

- Kato, M., Takehana, Y., Sakaizumi, M., and Hamaguchi, S. 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. 2009 September 16.

〔Review articles〕

- Kinoshita, M., Murata, K., Naruse, K., and Tanaka, M. (2009). Medaka: Biology, Management and Experimental Protocols. (Wiley-Blackwell, Ames, Iowa).
- Sasado, T. (2009). Artificial insemination using frozen medaka sperm. In: Medaka: Biology, Management and Experimental Protocols. Kinoshita, M., Murata, K., Naruse, K., and Tanaka, M. eds. (Wiley-Blackwell, Ames, Iowa), pp. 110-116.
- Sasado, T. (2009). Cryopreservation of medaka sperm. In: Medaka: Biology, Management and Experimental Protocols. Kinoshita, M., Murata, K., Naruse, K., and Tanaka, M. eds. (Wiley-Blackwell, Ames, Iowa), pp. 105-110.