LABORATORY OF BIORESOURCES

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Teleosts comprise about half of all vertebrate species and adapt 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. Construction of *Polypterus* Genomic DNA Library for Analysis of the Fish Genome Evolution

Comparative genomic analysis using medaka, zebrafish, *Tetraodon* and human as an outgroup revealed that the euteleost has experienced the teleost specific 3rd round genome duplication and the common ancestor of all euteleost should have 13 pre-duplicated proto-chromosomes. To verify this hypothesis, it is effective to compare the genomic structure of the reconstructed proto-chromosomes and fish chromosomes without the 3rd round genome duplication. One appropriate fish for this comparison is Polypteridae. We have started the construction of the genomic DNA library of *Polypterus senegalus* using fosmid vector and established the fosmid genomic library with 200,000 independent clones in 2008. In addition to this genomic resource, we are now establishing cell lines derived from the caudal fin of *Polypterus senegalus*.

Ⅱ. Evolution of the sex chromosome and sex determining 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

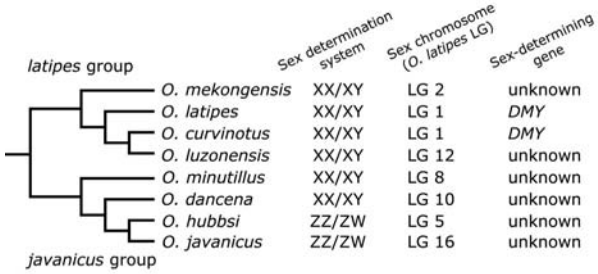

Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

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.

Ⅲ. Involvement of two chemokine receptors CXCR4b and CXCR7 in the regulation of the PGC migration

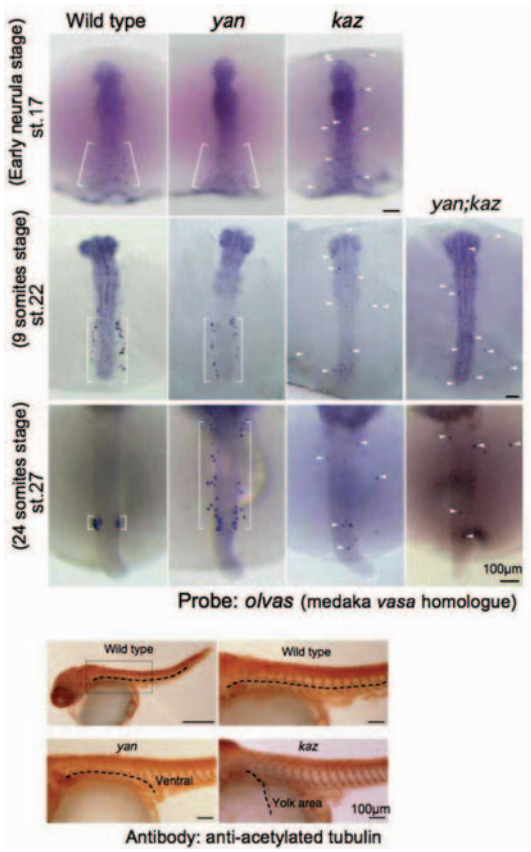

Figure 2. *yanagi* (*yan*) and *kazura* (*kaz*) mutations affect PGC migration and lateral line positioning.

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 teleost 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 (Figure 2). *kaz*$^{j2\text{-}15D}$ was identified as a missense mutation in chemokine receptor gene *cxcr4b* expressed in PGCs. Embryonic injection of *cxcr4b* mRNA with *olvas* 3'UTR rescued the PGC phenotype of *kaz* mutant, indicating a cell-autonomous function of *cxcr4b* in PGCs. *yan*$^{j6\text{-}29C}$ was identified as a nonsense mutation in the *cxcr7/rdc1* gene encoding another chemokine receptor. *cxcr7* transgene with genomic flanking sequences rescued the *yan* mutant phenotype efficiently at the G0 generation. *cxcr7* was expressed in somites rather than PGCs. *cxcr7*-expressing somitic domain expanded posteriorly with its margin immediately anterior of posteriorly drifting PGCs, as if PGCs were pushed toward the gonadal area. The *kaz* and *yan* mutants are also defective in the lateral line positioning, suggesting combined employment of these receptor systems in various cell migratory processes.

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

4-1 Full Length cDNA Sequencing Project

Although we published the draft genome sequence of medaka in 2007, the annotation of medaka genome is not yet well established. Sequencing of the full length cDNA clones is one of the most efficient methods for genome anotation. To do this, we made six full length cDNA libraries (developmental stage 22, 35, 40, ovary, brain and liver) and determined the sequences of both ends of 150, 000 clones in collaboration with the National Institute of Genetics' Kohara and Fujiyama labs. After mass alignment of all sequences, we found 16, 851 independent sequences. 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/).

4-2 Establishment of Core Facility of NBRP Medaka

In 2007, the 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 the integrated information on medaka (Figure 3). NBRP Medaka is aiming to establish a first rate biological resource with the highest possible levels of accessibility and ease of use.

Figure 3. NBRP Medaka website

Publication List

〔Original papers〕

- Ahsan, B., Kobayashi, D., Yamada, T., Kasahara, M., Sasaki, S., Saito, T. L., Nagayasu, Y., Doi, K., Nakatani, Y., Qu, W., et al. (2008). UTGB/medaka: genomic resource database for medaka biology. Nucleic Acids Res. *36*(Database issue), D747-D752.
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- Nakamura, S., Aoki, Y., Saito, D., Kuroki, Y., Fujiyama, A., Naruse, K., and Tanaka, M. (2008). Sox9b/sox9a2-EGFP transgenic medaka reveals the morphological reorganization of the gonads and a common precursor of both the female and male supporting cells. Mol. Reprod. Dev. *75*, 472-476.
- Nagai, T., Takehana, Y., Hamaguchi, S., and Sakaizumi, M. (2008). Identification of the sex-determining locus in the Thai medaka, *Oryzias minutillus*. Cytogenet. Genome Res. *121*, 137-142.
- Sasado, T., Yasuoka, A., Abe, K., Mitani, H., Furutani-Seiki, M., Tanaka, M., and Kondoh, H. (2008). Distinct contributions of CXCR4b and CXCR7/RDC1 receptor systems in regulation of PGC migration revealed by medaka mutants *kazura* and *yanagi*. Dev. Biol. *320*, 328-339.
- Takehana, Y., Hamaguchi, S., and Sakaizumi, M. (2008). Different origins of ZZ/ZW sex chromosomes in closely related medaka fishes, *Oryzias javanicus* and *O. hubbsi*. Chromosome Res. *16*, 801-811.