

## LABORATORY OF BIORESOURCES



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The medaka is a small egg-laying 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 the dynamics of transcriptional and chromatin accessibility landscape during medaka development, the understanding of pigment cell differentiation using body color mutants, genome sequence of the Javanese medaka, *Oryzias javanicus* and the molecular genetic basis of diversified sexually dimorphic traits in *Oryzias* species. In addition to these activities, our laboratory was charged with the responsibility of leading the National BioResource Project Medaka (NBRP Medaka) from 2007.

### I. Daynamic transcriptional and chromatin accesibility landscape during medaka development.

A high-quality genome sequence and a variety of genetic tools are available for medaka. However, existing genome annotation is still rudimentary, as it was mainly based on computational prediction and short-read RNA-seq data. To overcome this situations, we conducted long-read RNA-seq, short-read RNA-seq, and ATAC-seq coraboration with Dr. Tu at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. This work constructed a

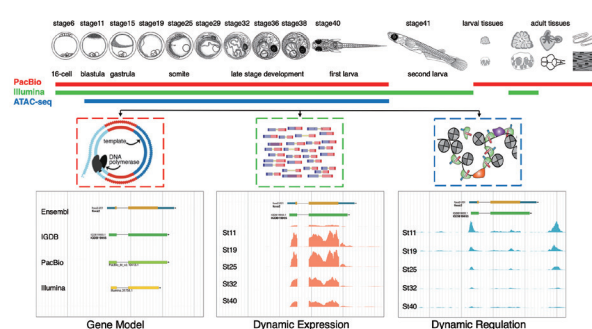


Figure 1. Overview of medaka multi-omics anslsysis during development

much-improved gene model set including about 17,000 novel isoforms and identified 1600 transcription factors, 1100 long non-coding RNAs, and 150,000 potential cis-regulatory elements as well. The work provides the first comprehensive omics datasets of medaka embryogenesis. The data portal ([http://tulab.genetics.ac.cn/medaka\\_omics](http://tulab.genetics.ac.cn/medaka_omics)) will serve as a daily reference tool for the entire medaka community.

### II. Identification of the causal gene of the medaka body color mutant, *few melanophore(fm)*

The body coloration of animals is due to pigment cells derived from neural crest cells which are multipotent and differentiate into diverse cell types. Medaka possess four distinct types of pigment cells known as melanophores, xanthophores, iridophores, and leucophores. The *few melanophore (fm)* mutant found amongst medaka is characterized by reduced numbers of melanophores and leucophores. We have identified *kit*-ligand as the gene whose mutation gives rise to the *fm* phenotype. This identification was confirmed by generation of *kit*-ligand knockout medaka and the findings that these fish also manifest reduced numbers of melanophores and leucophores and fail to rescue the *fm* mutant phenotype. We also found that expression of *sox5*, *pax7a*, *pax3a*, and *mitfa* genes is down-regulated in both *fm* and *kit*-ligand knockout medaka, implicating c-Kit signaling in the regulation of the expression of these genes as well as the encoded transcription factors in pigment cell specification.

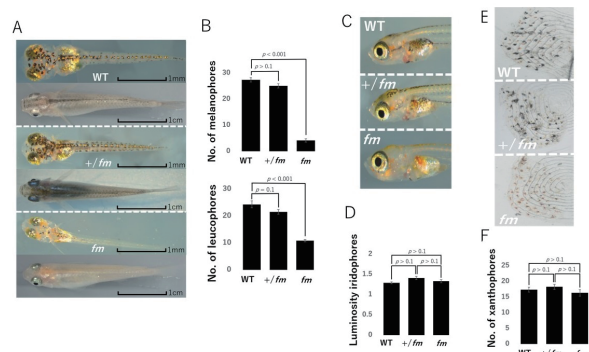


Figure 2. The *fm* mutants show a reduction in melanophore and leucophore pigmentation(A and B) and not in xanthophore and iridophore pigmentation (C, D, E and F).

### III. Genome sequence of the Javanese medaka, *Oryzias javanicus*, as a model for studying seawater adaptation

Medaka fish in the genus *Oryzias* are an emerging model system for studying the molecular basis of vertebrate evolution. This genus contains approximately 35 species and exhibits great morphological, ecological and physiological differences among it's species. Among these species, the Java medaka, *Oryzias javanicus*, is the species that has most typically adapted to seawater. We sequenced and assembled the whole genome of *O. javanicus*, as a model fish species for studying molecular mechanisms of seawater adaptation. In teleost fish, the major osmoregulatory organs are the gills, intestine and kidney, and these play

different roles to maintain body fluid homeostasis. Many genes encoding hormones, receptors, osmolytes, transporters, channels and cellular junction proteins are potentially involved in this osmotic regulation. In addition to the osmoregulation, hatching enzyme activity dramatically changes in different salt conditions. At the hatching stage, fish embryos secrete a specific cocktail of enzymes in order to dissolve the envelope. In the medaka *O. latipes*, digestion of the envelope occurs after the cooperative action of two kinds of hatching enzymes: (i) the high choriolytic enzyme (HCE) and (ii) the low choriolytic enzyme (LCE) (Yasumasu *et al.*, 2010). The HCE shows higher activity in freshwater than in brackish water (Kawaguchi *et al.*, 2013). Thus, availability of the high-quality reference genome in *O. javanicus* would facilitate further research for investigating the molecular basis of physiological differences including the osmotic regulation and the hatching enzyme activity among *Oryzias* species.

#### IV. Adrianichthyidae fish in Sulawesi: a model system to explore the molecular genetic basis of diversification in sexual dimorphism

Sexual dimorphism is prevalent, but often differs remarkably between closely related species. However, we know little about which genes and genetic changes can actually contribute to diversification of sexually dimorphic traits. The family Adrianichthyidae are a small teleost species commonly considered to be medaka. Although their native habitats are widely distributed in East and South-East Asia, 20 of 37 Adrianichthyidae species are endemic to Sulawesi, Indonesia. We have studied the molecular genetic basis of diversification in sexual dimorphism using the endemic species as a model system, because their sexual dimorphic body colorations are significantly diversified in closely-related species. As a research resource for the Sulawesi species, we firstly sequenced and assembled a genome of *Oryzias celebensis*. The assembly was anchored to 18 different chromosomes by linkage mapping and was annotated using RNA-seq data from adult and embryonic tissues. We then investigated the molecular mechanisms underlying red coloration in pectoral fins, a characteristic feature of *O. woworae* males. Quantitative trait loci (QTL) mapping in a F2 intercross between a male of *O. woworae* and a female of a close relative *O. celebensis* without any red fins revealed that an autosomal locus controls the red pigmentation. Subsequent quantitative gene expression analysis revealed that a gene is a strong candidate responsible for the red fins, which is highly expressed in the red fins of *O. woworae* males by the cis-regulatory mutation. CRISPR/Cas-mediated mutagenesis in the gene caused a deficiency in the fins' pigment cells. Additionally, a behavioral analysis showed that the mutant males had lower reproductive success than the wild-type males. These results suggest that ectopic expression of an autosomal gene in the pectoral fins by the cis-regulatory change will cause the red coloration in the pectoral fins, which might spread by increasing the reproductive success in males.

#### Publication List:

##### [Original papers]

- Amemiya, S., Hibino, T., Minokawa, T., Naruse, K., Kamei, Y., Uemura, I., Kiyomoto, M., Hisanaga, S., and Kuraishi, R. (2019). Development of the coelomic cavities in larvae of the living isocrinid sea lily *Metacrinus rotundus*. *Acta Zool.* 100, 414-430. doi: 10.1111/azo.12274
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- Stolper, J., Ambrosio, E.M., Danciu, D.-P., Buono, L., Elliott, D.A., Naruse, K., Martínez-Morales, J.R., Marciniak-Czochra, A., and Centanin, L. (2019). Stem cell topography splits growth and homeostatic functions in the fish gill. *eLife* 8, e43747. doi: 10.7554/eLife.43747
- Sugiyama, A., Scharl, M., and Naruse, K. (2019). Histopathologic features of melanocytic tumors in *Xiphophorus* melanoma receptor kinase (*xmrk*)-transgenic medaka (*Oryzias latipes*). *J. Toxicol. Pathol.* 32, 111-117. doi: 10.1293/tox.2018-0058
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##### [Review articles]

- Murata, K., Kinoshita, M., Naruse, K., Tanaka, M., and Kamei, Y. eds. (2020). *Medaka: Biology, Managements, and Experimental Protocols*, Volume 2. (Wiley-Blackwell, Iowa, USA). ISBN: 978-1-119-57529-0
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