

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. Dynamic transcriptional and chromatin accessibility landscape during medaka development.

A high-quality genome sequence and a variety of genetic tools are available for medaka. However, existing genome

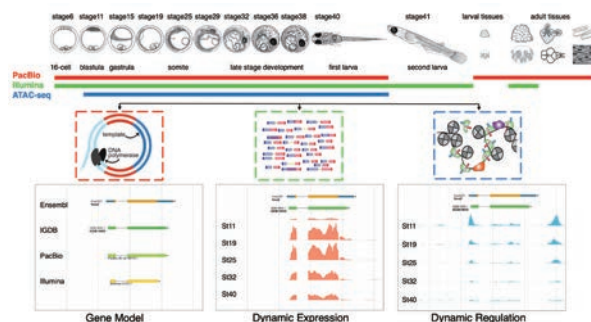


Figure 1. Overview of medaka multi-omics analysis during development

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 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) 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.

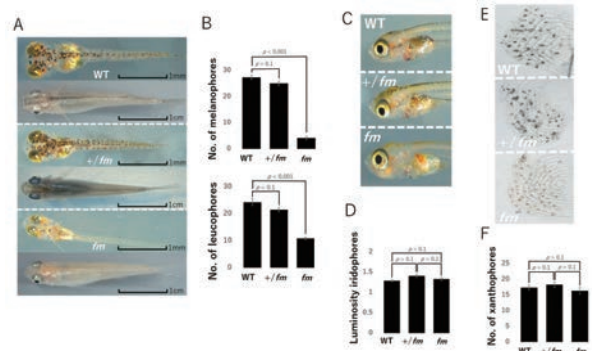


Figure 2. The *fm* mutants show a reduction in melanophore and leucophore pigmentation (A and B) and not in xanthophore and iridophore pigmentation (C, D, E and F).

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 its 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. National BioResource Project Medaka

Our laboratory has been acting as main facility of the Medaka BioResource Project (NBRP Medaka) in the fourth phase of NBRP, which started in 2017. We are providing wild strains, and related species, genomic resources (approximately 400,000 cDNA clones from 30 different cDNA libraries (containing approximately 23,000 different sequences) and BAC/Fosmid clones covering the entire medaka genome), and hatchery enzymes essential for embryo manipulation and live imaging. These bioresources are available on our website (Figure 3. <https://shigen.nig.ac.jp/medaka/>). These bioresources can be searched by various methods such as keywords, sequence homology, and expression profiles using the database on the website. In addition, genome browsers for the three inbred strains, phylogenetic relationships between wild strains of medaka and related species, and experiment manuals are also available. With the approval of the second supplementary budget for FY2020, we were able to install a cabinet-type fish tank washing machine. This has freed up our technical support staff from the need to wash the tanks by hand, and has allowed us to focus more on breeding and management, which requires more human work. In addition, a system to remotely monitor the temperature, humidity, and illumination in the medaka breeding rooms and the water temperature in the breeding tanks was installed. The air conditioner in the breeding room was also upgraded. We have continuously monitored the medaka breeding conditions using these systems.



Figure 3. NBRP Medaka website

Publication List:

(Original papers)

- Ansay, S., Mochida, K., Fujimoto, S., Mokodongan, D.F., Sumarto, B.K.A., Masengi, K.W.A., Hadiaty, R.K., Nagano, A.J., Toyoda, A., Naruse, K., *et al.* (2021). Genome editing reveals fitness effects of a gene for sexual dichromatism in Sulawesian fishes. *Nat. Commun.* 12. DOI: 10.1038/s41467-021-21697-0
- Li, Y., Liu, Y., Yang, H., Zhang, T., Naruse, K., and Tu, Q. (2020). Dynamic transcriptional and chromatin accessibility landscape of medaka embryogenesis. *Genome Res.* 30, 924–937. DOI: 10.1101/gr.258871.119
- Takehana, Y., Zahm, M., Cabau, C., Klopp, C., Roques, C., Bouchez, O., Donnadieu C., Barrachina, C., Journot, L., Kawaguchi, M., Yasumasu, S., *et al.* (2020). Genome sequence of the euryhaline javafish medaka, *Oryzias javanicus*: A small aquarium fish model for studies on adaptation to salinity. *G3: Genes Genomes Genet.* 10, 907–915. DOI: 10.1534/g3.119.400725