CENTER FOR THE DEVELOPMENT OF NEW MODEL ORGANISMS

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Since the beginning of life on Earth, living organisms have evolved to adapt to various environments, and have spawned a wide variety of species. Modern biological research has put an emphasis on elucidating the basic principles common to many species, and has progressed thanks to the intensive analysis of a limited number of species known as model organisms, which are easy to handle in a laboratory environment. However, this development has most likely left many interesting biological phenomena unexamined as their distinctive characteristics are observed only in a particular group of species. How we overcome this is an important challenge for biology hereafter.

To solve these problems, we must choose a species most suitable to analyze the phenomenon to be researched, and then establish it as a new model organism by developing methods using procedures that are necessary for modern biological analyses. These include stable raising, breeding and experimental manipulation techniques, analyses of the genome information and gene expression, and gene manipulation techniques using gene insertion and genome editing techniques.

To this end, The Center for the Development of New Model Organisms was established in 2013. Through its activities, organisms that have been out of reach of scientific research were designated as new model organisms. For example, we study aphids and sea anemones to understand the symbioses, and rhinoceros beetles to get insights into the sexual dimorphism. We are refining various techniques for studying targeted new model organisms ranging from genome analysis to genetic engineering to build seamless workflows that will be shared among the research community.

Research activity by S. Shigenobu

Professor Shuji Shigenobu is the principal investigator of the Laboratory of Evolutionary Genomics. Refer to the laboratory page for details.

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Two technical innovations have recently changed biology: Next generation sequencing (NGS) and Genome editing. NGS reveals whole genome sequences and gene expression profiles from various organisms. Genome editing accelerates the functional characterization of numerous genes involved in the phenomenon of life. Accordingly, we are now basically able to choose any organism which we are interested in, and carry out functional analyses by using these tools.

1-1 Development of genome editing techniques for various organisms.

Recent advances in the CRISPR-Cas system now allow for reverse genetics in various organisms. However, it has been hampered by the lack of a simple and efficient method for gene modification in most of the non-model organisms. To overcome this issue, we developed a highly-efficient workflow for gene knockout in the founder using this CIRSPR-Cas. We call the virtually knockout founders “crispants”. Crispant assay provides us with a practical and rapid tool for functional screening of numerous genes of interest beyond the post-genome era (Figure 1).

Despite the practical utility of the knockout technique, there is still room for improvement in the integration of exogenous DNA into a target chromosomal site (*i.e.* knock-in), which is still somewhat limited in various organisms. Therefore, we are currently developing more efficient and practical knock-in techniques than conventional ones.

Figure 1. *tyrosinase* crispant in *P. waltl*. A knock-out founder of tyrosinase, a melanin synthesis enzyme, and wild newt (left and right, respectively). *tyr* crispant shows full albinism.

1-2 Finding new model organisms and deciphering organ regeneration

One of our missions is to discover unique organisms and develop them as new model organisms for basic biology. A recent example of this is our recent establishment of the newt *Pleurodeles waltl* as an experimental model animal for regenerative biology using NGS and genome editing techniques. *P. waltl* possesses several excellent characteristics as a model animal: easy breeding, short sexual maturation period, remarkable regenerative capacity and comparatively high efficiency of genome editing (Figure 2). We are currently researching the molecular basis of organ regeneration using this newt. In addition, we widely support researchers who attempt to develop new model organisms contributing to the up-coming biology.

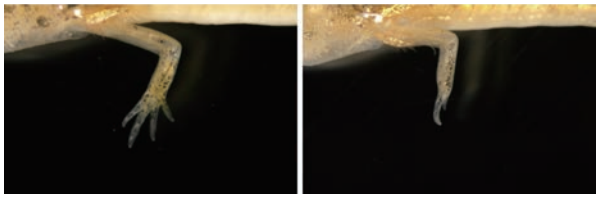

Figure 2. A limb-specific enhancer (ZRS/MFCS1) of *sonic hedgehog* crispant in *P. waltl*. Phenotypes of limb regeneration in wild and ZRS/MFCS1 crispant (left and right, respectively). Unlike in normal limb regeneration in the wild type, severe reduction of digit formation was seen in ZRS/MFCS1 crispant.

Publication List:

〔Original papers〕

- Habuta, M., Yasue, A., Suzuki, K.T., Fujita, H., Sato, K., Kono, H., Takayama, A., Bando, T., Miyaishi, S., Oyadomari, S., *et al.* (2020). Fgf10-CRISPR mosaic mutants demonstrate the gene dose-related loss of the accessory lobe and decrease in the number of alveolar type 2 epithelial cells in mouse lung. PLoS One *15*. DOI: 10.1371/journal.pone.0240333
- Iida, M., Suzuki, M., Sakane, Y., Nishide, H., Uchiyama, I., Yamamoto, T., Suzuki, K.T., and Fujii, S. (2020). A simple and practical workflow for genotyping of CRISPR-Cas9-based knockout phenotypes using multiplexed amplicon sequencing. Genes to Cells *25*, 498–509. DOI: 10.1111/gtc.12775
- Sanoh, S., Hanada, H., Kashiwagi, K., Mori Tsukasa and Goto-Inoue, N., Suzuki, K.T., Mori, J., Nakamura, N., Yamamoto, T., Kitamura, S., Kotake, Y., *et al.* (2020). Amiodarone bioconcentration and suppression of metamorphosis in Xenopus. Aquat. Toxicol. *228*. DOI: 10.1016/j.aquatox.2020.105623