NBRP Zebrafish News Letter March 2024

Thank you for your continued support of the National BioResource Project (NBRP)-Zebrafish. The second year of the fifth phase of the NBRP Zebrafish Research Project is almost over. We look forward to your cooperation in the coming fiscal year.

Users' Voices

Dr. Sachiko Tsuda of Saitama University gave us an article on small fish research and her comments on the NBRP Zebrafish.

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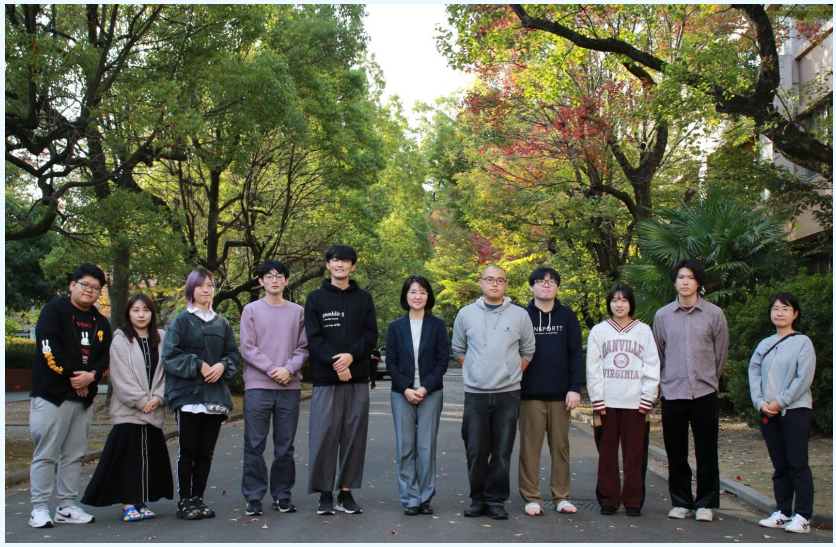


Laboratory Members

I am studying the function and development of cerebellar neural networks in small fish at the Graduate School of Science and Engineering, Saitama University. My first encounter with small fish dates back to 2002, when I entered the Department of Biological Sciences, Faculty of Science, The University of Tokyo, and received my first practical training from Professor Hiroyuki Takeda (Laboratory of Embryology), who had just arrived there. I had entered the zoology course with an interest in neural development, but the beauty of the transparent zebrafish embryo was so fascinating and I continued to visit the Takeda Lab after my internship. I earned my degree under the supervision of Prof. Takeda and became a postdoctoral researcher in the same laboratory. Since Takeda Lab has been using both medaka and zebrafish, my research began with the genetic screening of medaka mutants and the analysis of one of medaka novel mutants called *tacobo*, which shows abnormalities in its head morphology. We characterized the mutant phenotype with histological and cell biological analysis, including time-lapse imaging. The results revealed that *tacobo* encodes the extracellular matrix protein Laminin and its mutant exhibits abnormal neuronal differentiation caused by abnormal Interkinetic nuclear migration (INM), a process where the nucleus moves within the cytoplasm of elongated neuroepithelial progenitor cells depending on those cell cycle stages. This finding led us to propose a new mechanism for the formation of neural tube tissue by extrinsic factors. Since I had always wanted to study developmental phenomena from a cell biological perspective, it was fortunate to discover that the causative gene was a cell adhesion molecule and the phenotype was in the INM, in which I had long been interested. This experience made me realize that research can be hard but enjoyable. In particular, the Takeda Lab has a free-spirit atmosphere, allowing me to continue my thesis work according to my interests. I could enjoy creating my own research through trial and error. At that time, medaka was still in the early stage in terms of its standpoint as a model animal, more particularly its use as genomic, developmental and cellular biological experimental tool, but Irecall it was also a great benefit for me to have the feeling that I could try anything by myself.

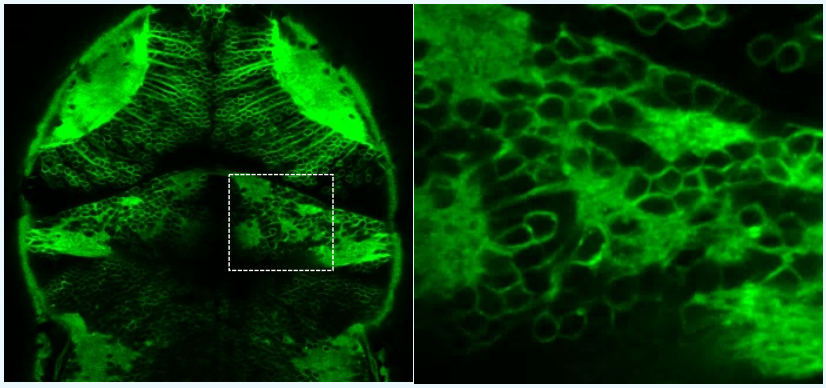
After obtaining Ph.D, I wanted to expand my study in the field of brain function which also contributes to the development of the neural system. I joined the laboratory of Prof. George Augustine (Duke-NUS Graduate Medical School Singapore), an expert in neurophysiology and optical technology. I also spent my summers as a researcher and Grass Fellow (young principal investigator) at the Marine Biological Laboratory in Woods Hole in the suburbs of Boston, and I was like a migratory bird, traveling and working with researchers from all over the world. I have made many friends I still connect with today. I decided to leave small fish and embryology for a while to major in

neurophysiology and photonics of the mouse cerebellum, an excellent system for circuit analysis. We combined electrophysiology of cerebellar Purkinje cells (patch-clamp recordings) with optogenetic stimulation to reveal the previously poorly understood spatiotemporal structure of inhibitory neuronal circuits in the cerebellum. It was quite an experience for me to set up the recording system alone in the George Lab in Woods Hole as soon as I had mastered patch-clamp recording in Singapore. It was a fresh sensation that was different from imaging. Now, I am performing electrophysiological recordings with zebrafish, and I feel that cells are similar across species.

In addition to these electrophysiological approaches, I also began voltage imaging using voltage-sensitive dyes, which enables direct and fast optical monitoring of the membrane potentials. I was lucky working closely with Dr. Larry Cohen, the pioneer of voltage imaging, being on the same floor, and found myself becoming a member of the voltage imaging community. Unlike today, when it has become fashionable, I was often criticized for using this technique with its low signal-to-noise ratio and high degree of difficulty, but I developed a microscope that could simultaneously perform optogenetic stimulation and voltage imaging and succeeded in performing the spatiotemporal analysis of inhibitory neural circuits in the cerebellum entirely with light.

As a side note, there is a replica of a letter from Dr. Katsuma Dan at the Misaki Marine Biological Station of the University of Tokyo, where I stayed as an undergraduate, in which he expressed his wish to preserve the research facilities when they were confiscated by the U.S. military after World War II. I heard that the letter was sent to Woods Hole Marine Biological Laboratory. During my first summer at Woods Hole, I was delighted and deeply moved to find the real letter displayed in front of the library as a bridge between Japan and the United States.

During my various fulfilling study abroad experiences, my love for fish and embryology remained unchanged. Fortunately, both Singapore and Woods Hole are places where fish and embryology research is flourishing, and I was able to follow the flow of the field from the outside. I was wondering if it was time to take a fresh look at developmental biology from a physiological perspective, when I was hired as a tenure-track assistant professor at Saitama University and was able to return to the world of small fishes again. I was also surprised by the opacity of the mouse brain slice and re-experienced the beauty of small fishes, which are transparent and easy to conduct various experiments, which I had taken for granted. With the support of many, including Professor Kyo Yamasu, I started a research group and we are now working on the development of the cerebellar neural network using zebrafish. During development, Purkinje cells show active and diverse population activities, which can be analyzed in detail by voltage imaging. By introducing protein-type voltage sensors (genetically encoded voltage indicators), which have been actively developed in recent years, into zebrafish, we have succeeded in the long-term recording of neuronal populations in the spinal cord and cerebellum and are observing the emergence process of membrane potential fluctuations. In addition to the dynamics of cellular populations, we are also expanding our view to the behavior of fish populations (schooling, collective behavior). We are trying to understand neural mechanism underlying schooling behavior of fish and the involvement of the surrounding environment in which they grow by using optical technology, embryological analysis, and physiological study. I feel that small fishes present a low hurdle for the introduction of various technologies, and it is easy to conduct research using multidisciplinary approaches. I would like to clarify the function of cells and individuals as a group and the mechanisms of their formation by taking advantage of small fishes and the diversity and uniqueness of fishes.



Transgenic zebrafish expressing a voltage sensor ASAP1.
An enlarged view of the cerebellar region (right).

Saitama University is located in the metropolitan area, surrounded by lots of greenery. It has a pleasant environment where we can concentrate on our research while listening to the sounds of birds and trees on the campus. Last year, we had the opportunity to hold a small fish research meeting at the Saitama University campus, so many of you may have enjoyed it. Please contact us if you have opportunities to drop by. We are currently looking for enthusiastic students and post-doc researchers as well.

Lastly, the NBRP has been indispensable in helping us to launch our research. The NBRP has provided us with a variety of zebrafish strains, preserved newly established strains, and responded to requests from both domestic and overseas researchers. I hope to contribute to the small fish community, which has nurtured me as a researcher through the NBRP and related programs. I look forward to your continued guidance and encouragement.

New Strains

We are pleased to present the newly deposited and released strains as a resource.
You can order strains from our website (https://shigen.nig.ac.jp/zebra/).

Fads2
Tg(fli1a:EGFP-toca1) nf10Tg
Tg(5xUAS:mCherry) ncv76Tg
TgBAC(dcn:tagBFP) ncv546Tg
TgBAC(cxcr4a:dEGFP) ncv548Tg
Tg(sox9a:opt creERt2; crysaa:EGFP) tyt229
Tg(sox9a:opt creERt2; crysaa:EGFP) tyt230
Tg(krt18:creERt2; crysaa:EGFP) tyt236 ;
Tg(Ola.Actb:LOXP-DsRED2-LOXP-EGFP) tyt201
Tg(E5-0.7k:EGFP) tyt220
Tg(hsp70l:dnLef1) tyt225
Tg(krt18:mcherry) tyt237
Tg(5xUAS-hsp70l:GtCCR4-EGFP,
myl7:mCherry) nub88Tg
Tg(tetO:Had.Rh1-Flag-P2A-TagCFP,
myl7:mCherry) nub65Tg
Tg(tetO:parapinopsina-Flag-P2A-TagCFP,
myl7:mCherry) nub69Tg
Tg(tetO:bPAC-MT-T2A-tDimer,
myl7:mCherry) nub79Tg
keap1a it302 ; keap1b it308
cers2b+1
cers2bΔ2
sting+1
cgasΔ4
Tg(aldoca:BirA-2A-mCherry) nub105Tg
Tg(aldoca:skor1b-AVI-2A-BirA-2A-
mCherry) nub108Tg
Tg(aldoca:skor2-AVI-2A-BirA-2A-
mCherry) nub109Tg
foxp2 ex9+19/nub112
znf385c ex2+4/nub117
foxp1a ex2-4A/nub110
foxp2 ex9+11/nub113
foxp2 ex9-13/nub114
rora ex2-10/nub115
ebf1a ex1-8/nub116
ssx2ipa ex3-4/nub118
trpm7 agu75
dmd agu83
slc16a3 agu88
slc16a3 agu89
chr22del agu91
tial1 agu92
tial1 agu93
tial1 agu94
neurod2 ex2-7
barhl1a ex1-10
barhl1b ex1+5
tlx3b ex1-7
olig2 ex2-5
dnaseIIΔ4
dnaseIIΔ8
Tg(UAS:Xsu(H)DN) chi11
sidkey188i13.11 agu90
slc12a5a; slc12a5b agu98
zgc56095 agu100
nlk1 isi35
nlk1 isi35 ; nlk2 TALEN-B isi13
tlx3a -14
tlx3a -11
TG (β-actin:EGFP) ; mpv17 a9
nr2f1a sud-n1
nr2f1b sud-n3

Call for Deposit

- Would you like to be a depositor?-

RIKEN Center for Brain Science (RIKEN CBS) is Core center for zebrafish strain conservation and has a facility that can accommodate 200,000 fish. We are willing to accept deposits of strains from external research institutions, so please do not hesitate to deposit your strains with us. When depositing, the intellectual property rights of the depositor are protected.

- Sperm cryopreservation is available-

Sperm cryopreservation is the best way to back up your strain. Send us your fish and we will process and preserve a sample as soon as possible. We recommend 3-5 healthy male fish for processing, which will provide enough sperm for 45 IVFs.
Frozen sperm samples are backed up and stored at RIKEN CBS and National Institute for Basic Biology to prevent loss in disasters. We will be able to respond to your needs as much as possible.
Please feel free to contact us for more information. We look forward to working with you.

Organizations

NBRP Zebrafish is conducted by the following three institutes.

- Core center-
RIKEN Center for Brain Science
Principal Investigator Hitoshi Okamoto

-Sub-Core center-
National Institute of Genetics
Principal Investigator Koichi Kawakami

-Sub-Core center-
National Institute for Basic Biology
Principal Investigator Shin-ichi Higashijima

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