

LABORATORY FOR BIOTHERMOLOGY



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Temperature is an important parameter for living organisms. Cell activity is affected by temperature since the reaction ratio and stability of molecules, especially proteins in cells, depends on it. The environmental temperature which organisms function in vary, and the biological system that regulates body temperature, homeostasis, differs among various organisms. Furthermore, temperatures in either cells or at the molecular level have not been widely discussed to date. Nano-scale thermometers utilizing fluorescent proteins and dyes have been developed by numerous research groups including our own, and heterogeneity of temperatures in the micro environments of living cells have been reported on by said groups (Okabe *et al*, Nat Commun 2014, Kiyonaka *et al*, Nat. Methods 2014, and Nakano *et al*, PLoS One 2017). However, the meaning and mechanisms involved in single cells remain unclear. Therefore, our group is embarking upon a new research field, biothermology, by investigating the nature of temperature in living organisms ranging from nano to macro.

Our research group employs various and original biothermological research technologies. One of these is the infrared (IR) laser application, which enables single-cell or subcellular local heating by focusing IR through an objective lens on a microscope. This technology was originally developed for laser induced gene expression in the targeting of single-cells through the heat shock response, IR-LEGO: InfraRed Laser Evoked Gene Operator (Kamei *et al*, Nat. Methods 2009) (Figure 1). This local heating technique can be used

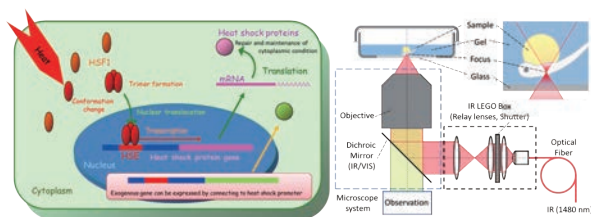


Figure 1. Schematic illustration of heat shock response in cells (left) and an infrared laser-evoked gene operator (IR-LEGO) microscope system.

to analyze the thermodynamics of a cell via the use of a nano-scale thermometer; the second of our abovementioned original techniques. To analyze temperature distribution at subcellular resolutions, a nano-scale thermometer is required. For this reason, we have focused on fluorescent proteins, and developed a genetically encoded thermometer, the so called gTEMP, with Dr. Takeharu Nagai from Osaka University (Nakano *et al*, PLoS One 2017). This thermometer has some unique properties, such as rapid responsiveness and applicability to wide temperature range. By utilizing this thermometer, we are developing a high-speed thermal imaging microscope system combined with IR irradiation optics. Through the analysis of thermal dynamics in cells, we are trying to reveal how temperature heterogeneity is generated within a single cell as well as considering its meaning from a biological standpoint.

Furthermore, our group is trying to improve the IR-LEGO technique. We initially applied this system to many organisms including animals (*i.e.* medaka, nematode, flies and frogs) and plants (*i.e.* *Arabidopsis* moss in collaborative research projects in cooperation with other laboratories. Because the heat shock response (HSR) is a transient response, gene expressions after IR-LEGO operation are also transient. Despite this, some of our collaborators wanted to realize cell fate mapping, which requires a long-term gene expression after IR irradiation. Accordingly, we employed the Cre/LoxP recombination system. As shown in Figure 2, we established a cre driver line, which possesses a heat shock promoter and loxP effector line which can permanently label irradiated cell lineages by utilizing fluorescent proteins. By using these transgenic lines, evidence was provided that indicated that exoskeletal tissues in the trunk region came from the mesoderm (Shimada *et al*, Nat Commun. 2013).

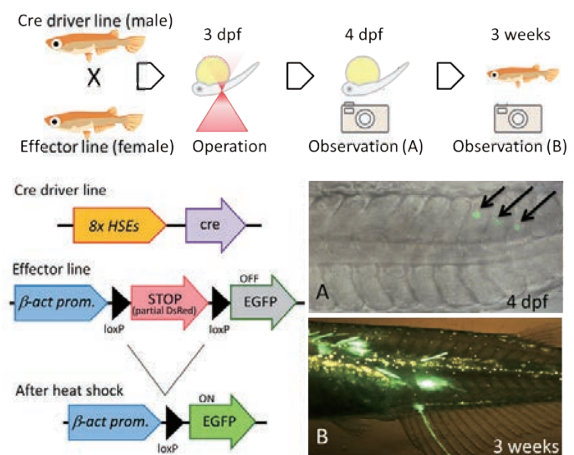


Figure 2. Long-term gene expression using the cre/loxP recombination system and an example of a practical experiment in medaka ranging embryos to adults.

The HSR is a conservative stress response system found in almost all organisms. In contrast, organisms live in various temperatures, and HSR essentially is the upper limit of habitable temperature range for each organism. This means that the preset temperature of HSR may differ among organisms. A key factor of this limitation is heat shock factor 1 (HSF1); a homologue within each organism. By substituting HSF1,

it may be possible to control the preset temperature of HSR, and this substitution could be applied to the improvement of HSR efficiency in IR-LEGO technology. We are now trying to lower the HSR temperature of medaka by substituting Japanese flounder HSF1. The HSF1 substitution provides us with various types of significant information and evidence related to molecular evolution and adaptation to environmental temperature. Thus, this is how this project also contributes to the study of biothermology.

We also promote other collaborative studies using microscopic techniques. One is a “clear observation project” for deep seeing into living organisms using adaptive optics (AO), which have been thoroughly developed in the field of astronomy as a key technology found in large telescopes, such as the Subaru telescope in Hawaii. Although observation using Earth based telescopes may be interfered with due to fluctuations in the atmosphere, AO can mitigate this. However, living materials have particular refractive indexes, so some organelles may hinder the ideal optical path for microscope observation, which is similar to the situation that exists regarding the atmosphere and telescopes. AO can also compensate for this disturbance by sensing and correcting wave fronts by using a wave front sensor and deformable mirror. Hence, we have developed a custom-made wide-field microscope equipped with an AO system for the observation of living organisms in collaboration with Dr. Yosuke Tamada from Utsunomiya University and Dr. Yutaka Hayano from the National Astronomical Observatory of Japan (NAOJ). By utilizing this microscopic system, we have successfully acquired high-resolution bright field and fluorescent images of living cells. Furthermore, AO can also be applied to IR-LEGO technology to improve IR energy focusing of irradiation in a manner similar to improving the sight of living organisms.

Publication List:

[Original papers]

- Ashida, Y., Honma, Y., Miura, N., Shibuya, T., Kikuchi, H., Tamada, Y., Kamei, Y., Matsuda, A. and Hattori, M. (2020). Imaging performance of microscopy adaptive-optics system using scene-based wavefront sensing. *J. Biomed. Optics*-25, 123707. DOI: 10.1117/1.jbo.25.12.123707
- Kanazawa, N., Goto, M., Harada, Y., Takimoto, C., Sasaki, Y., Uchikawa, T., Kamei, Y., Matsuo, M. and Fukamachi, S. (2020). Changes in a cone opsin repertoire affect color-dependent social behavior in medaka but not behavioral photosensitivity. *Front. Genet. 11*. DOI: 10.3389/fgene.2020.00801
- Kondow, A., Ohnuma, K., Kamei, Y., Taniguchi, A., Bise, R., Sato, Y., Yamaguchi, H., Nonaka, S. and Hashimoto, K. (2020). Light-sheet microscopy-based 3D single-cell tracking reveals a correlation between cell cycle and the start of endoderm cell internalization in early zebrafish development. *Dev. Growth Differ. 62*, 495-502. DOI: 10.1111/dgd.12695
- Nishiike, Y., Miyazoe, D., Togawa, R., Yokoyama K., Nakasone, K., Miyata, M., Kikuchi, Y., Kamei, Y., Todo, T., Ishikawa-Fujiwara, T., Ohno, K., *et al.* (2021). Estrogen receptor 2b is the major determinant of sex-typical mating behavior and sexual preference in medaka. *Curr. Biol. 31*, 1699+. DOI: 10.1016/j.cub.2021.01.089
- Watanabe, Y., Okuya, K., Takada, Y., Kinoshita M., Yokoi, S., Chisada, S., Kamei, Y., Tatsukawa, H., Yamamoto, N., Abe, H., Hashimoto, H., *et al.* (2020). Gene disruption of medaka (*Oryzias latipes*) orthologue for mammalian tissue-type transglutaminase (TG2) causes movement

retardation. *J. Biochem. 168*, 213–222. DOI: 10.1093/jb/mvaa038

- Zeng, C.-W., Kamei, Y., Shigenobu, S., Sheu, J.-C., and Tsai, H.-J. (2021). Injury-induced Cav1-expressing cells at lesion rostral side play major roles in spinal cord regeneration. *Open Biol. 11*. DOI: 10.1098/rsob.200304

[Review article]

- Abe, G., Hayashi, T., Yoshida, K., Yoshida, T., Kudoh, H., Sakamoto, J., Konishi, A., Kamei, Y., Takeuchi, T., Tamura, K., *et al.* (2020). Insights regarding skin regeneration in non-amniote vertebrates: Skin regeneration without scar formation and potential step-up to a higher level of regeneration. *Semin. Cell Dev. Biol. 100*, 109–121. DOI: 10.1016/j.semcdb.2019.11.014