Laboratory of Regeneration Biology

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Comparative Regenerative Biology

We use animals that demonstrate a high ability in regenerating body parts, such as planarians and newts, to understand the principle of regeneration. In particular, we investigate the difference between regenerative and non-regenerative animals to evoke said abilities from non-regenerative animals. We have already succeeded in achieving this with planarians, which were able to regenerate their heads through RNAi (Umesono *et al*., 2013 Nature), and accomplishing functional joint regeneration in frogs through the activation of reintegration systems (Tsutsumi *et al*., 2016 Regeneration).

We are currently trying to induce limb regeneration abilities in frogs, as they lose the capability to achieve complete limb regeneration after metamorphosis. We are now focusing on the *Sonic hedgehog* (*Shh*) super-enhancer MFCS1 (mammals-fishes conserved sequence 1), since it has been suggested that the loss of MFCS1 activity after metamorphosis might cause a failure in achieving the aforementioned limb regeneration in adult frogs (Yakushiji *et al*., 2009). When we compared the MFCS1 sequences between newts and frogs, newts were found to possess several specific sequences (Figure 1), suggesting that sequence differences might affect super-enhancer formation between newts and frogs after metamorphosis.

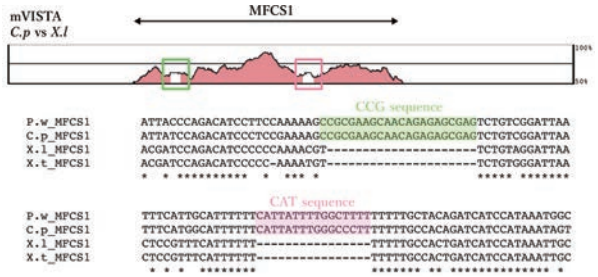

Figure.1 Comparison of the MFCS1 sequences between newts and frogs.

We subsequently tried to detect enhancer RNA (eRNA) which might be transcribed in the MFCS1 region. An interesting aspect of this was that eRNA was detected in regenerating blastema of adult newt from st. 2.0 (*Pleurodeles waltl:* Figure 2A).

Conversely, expression of eRNA was suppressed in the frog's blastema (*Xenopus laevis)* after metamorphosis (Figure 2B). Thus, we subsequently planned to swap the MFCS1 sequences between newts and frogs using CRISPR/Cas9 technology, expecting that the eRNA will be transcribed from the newt's MFCS1 after swapping in frogs.

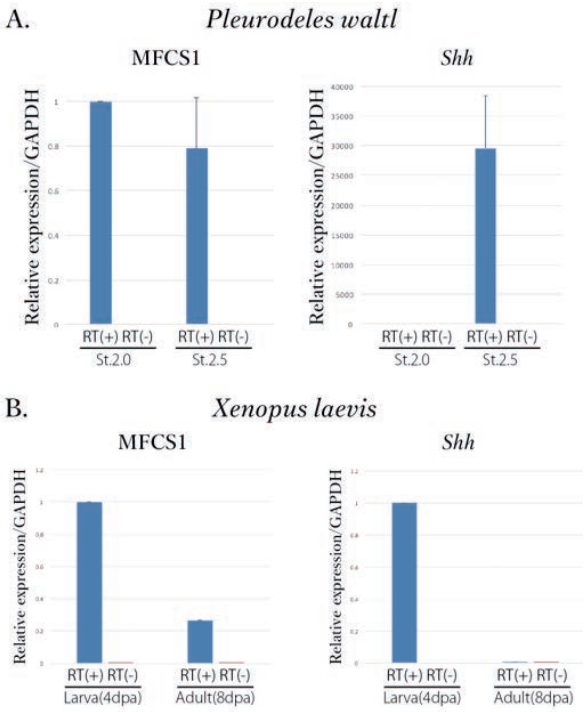

Figure.2 Expression of MFCS1-eRNA and *Shh* mRNA in the regenerating blastema of newt (*P.w.)* and frog (*X.l.)*
(A) The left and right panels show expression levels of MFCS1-eRNA and *Shh* mRNA, respectively. The left and right graphs in each panel were obtained from regenerating blastema at St.2.0 and St.2.5 of adult newts after amputation.
(B) The left and right panels shows expression levels of MFCS1-eRNA and *Shh* mRNA in frogs before and after metamorphosis.

Trials in isolating viable adult pluripotent stem cells derived from planarian using FACS

We have tried to develop an isolation method for viable adult pluripotent stem cells (aPSC) from planarians using FACS and succeeded in conditioning the low toxic staining method with both nuclear and cytoplasimic fluorescence dyes. 1μM Hoechst 33342 or 0.05 μg/ml Calsein AM could be used for isolating viable aPSC of the planarian, *Dugesis japonica*. We are presently investigating the effects of FGF- and Wnt-morphogens to cultured aPSC to demonstrate "the double gradient hypothesis", which was proposed by Thomas Hunt Morgan (Morgan, 1901) and our group (Umesono *et al*., Nature, 2013).

Publication List:

〔Original papers〕

- Auwal, M.A., Kashima, M., Nishimura, O., Hosoda, K., Motoishi, M., Kamimura, A., Okumura, A., Agata, K., and Umesono, Y. (2020). Identification and characterization of a fibroblast growth factor gene in the planarian *Dugesia japonica*. Dev. Growth & Differ. *62*, 527–539. DOI: 10.1111/dgd.12696
- Hijioka, M., Ikemoto, Y., Fukao, K., Inoue, T., Kobayakawa, T., Nishimura, K., Takata, K., Agata, K., and Kitamura, Y. MEK/ERK signaling regulates reconstitution of the dopaminergic nerve circuit in the planarian *Dugesia japonica*. Neurochem. Res. DOI: 10.1007/s11064-020-03226-5
- Miura, S., Tsutsumi, R., Agata, K., and Endo, T. (2020). Maturating articular cartilage can induce ectopic joint-like structures in neonatal mice. Regen. Eng. Transl. Med. *6*, 373–382. DOI: 10.1007/s40883-020-00176-w
- Sonpho, E., Wootthichairangsan, C., Ishida, M., Inoue, T., Agata, K., Maleehuan, A., Charngkaew, K., Chomanee, N., Moonsom, S., Wongtrakoongate, P., *et al*. (2020). ECM-body: A cell-free 3D biomimetic scaffold derived from intact planarian body. Zoolog. Sci. *37*, 307–313. DOI: 10.2108/zs190135

〔Review article〕

- Kashima, M., Agata, K., and Shibata, N. (2020). What is the role of PIWI family proteins in adult pluripotent stem cells? Insights from asexually reproducing animals, planarians. Dev. Growth & Differ. *62*, 407–422. DOI: 10.1111/dgd.12688