THE INTERNATIONAL JOURNAL OF
DEVELOPMENTAL
BIOLOGY
www.intjdevbiol.com

SUPPLEMENTARY MATERIAL

corresponding to:

Molecular mechanisms of limb regeneration: insights from regenerating legs of the cricket *Gryllus bimaculatus*

TETSUYA BANDO, TARO MITO, YOSHIMASA HAMADA, YOSHIYASU ISHIMARU,
SUMIHARE NOJI and HIDEYO OHUCHI*

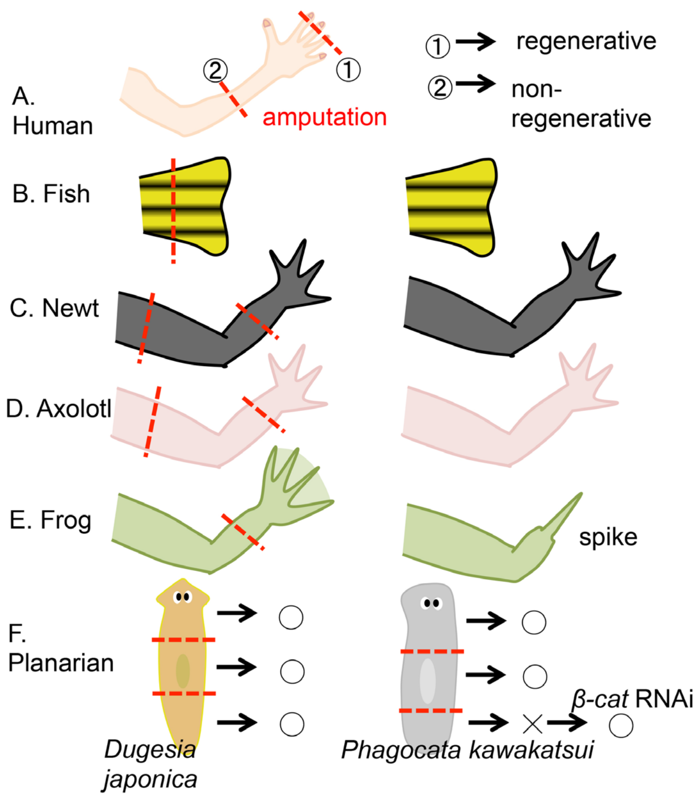
***Address correspondence to:** Hideyo Ohuchi. Department of Cytology and Histology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata-cho, Kita-ku, Okayama City, Okayama 700-8558, Japan. Tel:+81-86-235-7083. Fax:+81-86-235-7079. E-mail: hohuchi@okayama-u.ac.jp
https://orcid.org/0000-0003-1961-606X - **#Present address:** Division of Molecular Biology, Institute for Genome Research, Tokushima University

Full text for this paper is available at: http://dx.doi.org/10.1387/ijdb.180048ho

Diversity of regenerative ability in animals.

The regenerative capacity of mammalian species, such as *Homo sapiens* and *Mus musculus*, is limited to blood, newborn apical digits, epithelia and liver (see, e.g., (Yun, 2015) (Fig. S1A). By contrast, regenerative animals, including fish, newt, axolotl, frog, planarian, and insects, can regenerate lost parts of tissues (Figs. S1 B-F). The difference in regenerative ability may depend on the epigenetic states of genes involved in pattern formation. It might be that chromatin structural flexibility via epigenetic regulation is needed when differentiated cells in pre-existing tissues dedifferentiate into blastemal cells and leg-patterning genes must be re-expressed in regenerating legs. For example, the African clawed frog *Xenopus laevis* shows a varying regenerative capacity. In the tadpole stage, the amputated hindlimb is restored with digits, whereas it is regenerated as a spike-like structure in the froglet stage (Fig. S1E). In contrast, urodele newts or axolotls can regenerate complete limb, tail, heart, and eye lens structures, regardless of the amputated stage, from larva to adult. This difference in regenerative capacities between anurans and urodeles is due to the methylation status of the enhancer region of *Shh*, which is essential for limb development and regeneration (Yakushiji *et al.*, 2007; Katsuyama and Paro, 2011).

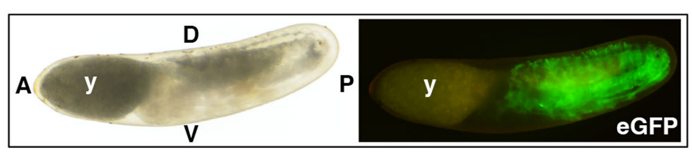
More recently, acetylation of the 9th lysine residue of histone H3 (histone H3K9) was found to contribute to tail regeneration in *Xenopus* (Suzuki *et al.*, 2016). Moreover, whereas the regenerative capacity of planarian *Dugesia japonica* is considerable, that of another planarian, *Phagocata kawakatsui*, is limited. By changing gene expression of *Phagocata* to that of *Dugesia*, the caudal fragment of *Phagocata* elicits regenerative capacity (Umesono *et al.*, 2013) (Fig. S1F). These results imply that although the regenerative capacity of human is limited, epigenetic regulation might recall the regenerative capacity, even for nonregenerative animals.



Supp. Fig. S1 (left). Diversity of regenerative ability in regenerative and non-regenerative animals. (A) *Mammals (e.g., human, mice) show the partial regenerative ability and can only regenerate some internal organs and distal tips.* **(B)** *Fish can regenerate fins and heart.* **(C,D)** *Newt and salamander can regenerate lost structures, such as limb, heart, tail, and eye lens, in adult.* **(E)** *Tadpoles can regenerate complete limbs, whereas frogs can regenerate rod-like "spike" structures.* **(F)** *Planarian* Dugesia japonica *can regenerate a whole body from head, middle (prepharyngeal and pharyngeal), and tail fragments. Planarian* Phagocate kawakatsui *cannot regenerate head regions from tail fragments, but can regenerate head regions after* β-cat *RNAi.*

Genome editing method with the CRISPR/Cas9 system can be used in the cricket.

Protocols for genome editing methods for the cricket were published (Wilson-Horch *et al.*, 2017). As an example, gene knock-in in cricket embryos with the CRISPR/Cas9 system is shown (Supp. Fig. S2). The enhanced GFP (eGFP) gene expression cassette was integrated into the cricket genome by using CRISPR/Cas9 system (personal information from Mito *et al.*).



Supp. Fig. S2 (right). Gene knock-in in cricket embryos with the CRISPR/Cas9 system. *Lateral views of a cricket embryo are shown. Left, bright field. Right, dark field. eGFP expression is seen in most of embryonic cells of the founder generation. A, anterior; D, dorsal; P, posterior; V, ventral; y, yolk.*