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Okayama University research: Understanding insect leg regeneration

(Okayama, 22 March) **Researchers at Okayama University report in *Development* the biomolecular mechanisms underlying leg regeneration in the two-spotted cricket, an insect capable of regrowing a leg following amputation.**

In organisms, tissue lost by a wound can be partially restored — and sometimes even completely. The ability to regenerate tissue varies across animal species. The regenerative abilities of humans are limited, whereas those of certain insects are such that they can regrow limbs. The latter is possible because at a wound, these insects can form a blastema, a group of special cells that can proliferate and differentiate, that is, change into different cell types. In order to better understand tissue regeneration, it is important to identify the biomolecular mechanisms that govern blastema formation. Now, Senior Lecturer BANDO Tetsuya and OKUMURA Misa (graduate students), Professor OHUCHI Hideyo from Okayama University and colleagues have performed experiments on leg regeneration in the two-spotted cricket (*Gryllus bimaculatus*), and found which genes and associated signaling pathways play a role in leg regrowth in this type of cricket.

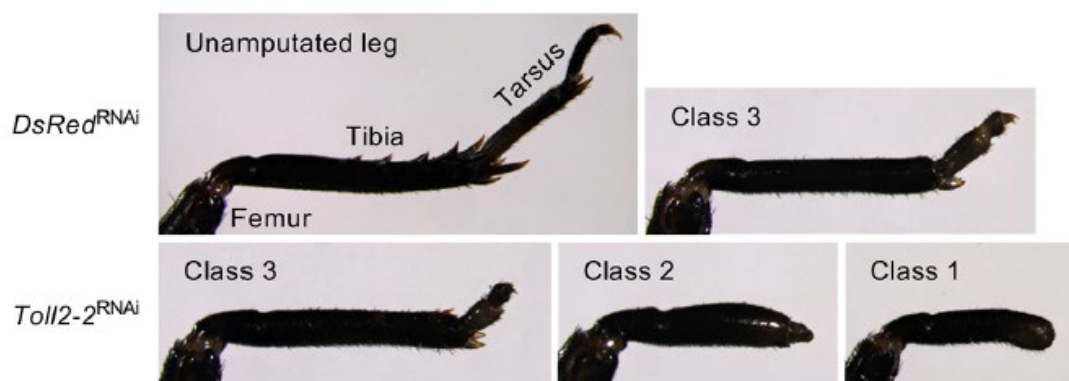
The scientists first looked at the effect of amputating a leg of a two-spotted cricket on gene expression (the production of biomolecules, typically proteins, encoded in the gene). They noted that certain genes from a set known as Toll genes displayed significant upregulated expression for up to 48 hours after leg amputation. Senior Lecturer BANDO and colleagues then applied a technique called RNA interference (RNAi), a gene ‘silencing’ procedure with which the production level of biomolecules associated with a specific gene can be reduced in a controlled way. Applying RNAi targeted at various Toll genes resulted in regrown but sometimes malformed legs, showing how genes affect the phenotype — in this case, leg morphology. From these experiments, it became clear that two genes, labeled Toll2-1 and Toll2-2, play a central role in blastemal formation and leg regeneration.

Further experiments showed that Toll2-2 regulates the accumulation of so-called plasmatocytes into the blastema that forms in the wound region. Plasmatocytes are insect macrophages, cells that can ‘eat’ harmful bacteria or organisms, and as such are an important part of an insect’s immune system. Apart from neutralizing ‘intruders’, macrophages help temper inflammation reactions by releasing cytokines. The latter are relatively small proteins that play important roles in cell signaling — the collective of processes that underlies the communication between different parts of an organism. The researchers found that the Toll2-2-regulated plasmatocyte accumulation triggers, through the release of cytokines, a signaling pathway that ultimately results in the blastemal cell proliferation leading to cricket leg regeneration.

The findings of Senior Lecturer BANDO and colleagues regarding the two-spotted cricket are relevant for the wider class of hemimetabolous insects (insects that do not have a pupal stage in their development, but rather transform gradually from egg to nymph to adult.) Quoting the scientists: “this study provides new insights into the function of Toll-related signaling for leg regeneration via plasmatocytes.”

Background

Regeneration: Biological regeneration refers to the process of restoring damaged or lost tissue. It makes individual cells and whole organisms resilient to natural perturbations or wounding. All species, from bacteria to humans, are capable of regeneration, but to different extents — regeneration can be complete or incomplete. Among the vertebrates, newts and salamanders have high regeneration capabilities; they can indeed regenerate various body parts including eyes, jaws, limbs and tails. Insects also display regeneration; the class of hemimetabolous insects (not undergoing a pupal stage during development) can regrow limbs, but only until the final molt (molting refers to the casting off of an outer layer during development). Senior Lecturer BANDO Tetsuya from Okayama University and colleagues have now investigated limb regeneration in the two-spotted cricket, and identified the genes and the signaling pathways that promote leg regeneration in this type of hemimetabolous insect.



Figure

Different phenotypes of regenerated legs of two-spotted crickets after RNA interference against Toll genes.

Reference

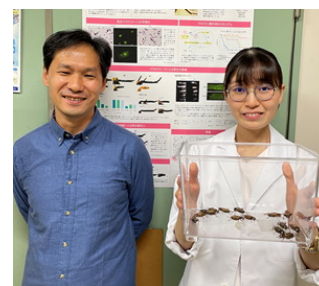
Tetsuya Bando, Misa Okumura, Yuki Bando, Marou Hagiwara, Yoshimasa Hamada, Yoshiyasu Ishimaru, Taro Mito, Eri Kawaguchi, Takeshi Inoue, Kiyokazu Agata, Sumihare Noji, Hideyo Ohuchi. Toll signalling promotes blastema cell proliferation during cricket leg regeneration via insect macrophages. *Development*. 2022, 149 (8): dev199916.

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<https://journals.biologists.com/dev/article-abstract/149/8/dev199916/273331/Toll-signalling-promotes-blastema-cell?redirectedFrom=fulltext>

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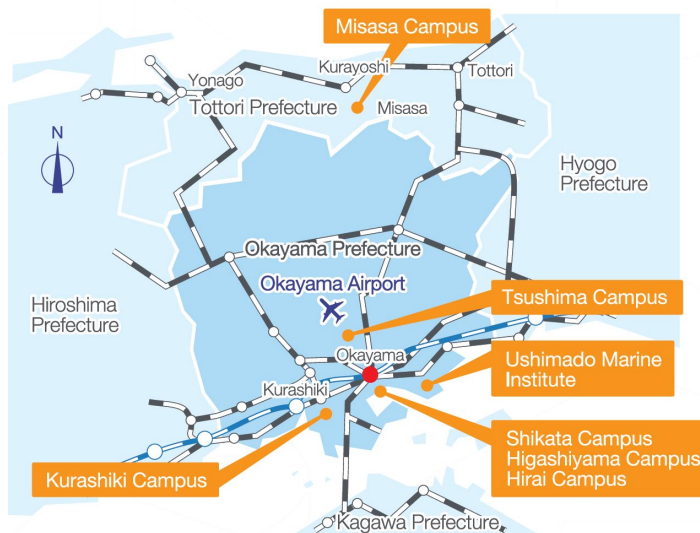
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Okayama University is located in the heart of Japan approximately 3 hours west of Tokyo by Shinkansen.

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