DIVISION OF EVOLUTIONARY BIOLOGY



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I. Evolution of Complex Adaptive Characters

The theory of natural selection and the neutral theory of molecular evolution are powerful concepts in evolutionary biology. However, even with such theories existing, unexplained phenomena still remain, one of which is the evolution of complexity. It is difficult to explain the mechanisms needed to evolve complex adaptive traits at the cellular and organismal levels, such as cell division machinery, regeneration, and novel organ development. Such traits are comprised of many components and become adaptive only when all of them are gathered together. However, based on evolutionary theory, each component should evolve one by one according to the accumulation of mutations. We aim to reveal the genetic networks regulating these complex traits and to identify the mechanisms needed for the evolution of complex characters.

II. Spatiotemporal regulation of the cell division axis as a grand plan of plant developmental evolution

The cell division axis must be properly regulated during the development of both metazoans and land plants. Genetic changes in the regulation of the cell division axis lead to the development of multicellular organisms. Since they do not have centrosomes and asteroid bodies, both of which are involved in the axis formation of metazoans, land plants most likely have different regulatory mechanisms. We aim to investigate the connecting factors between microtubules and GRAS transcription factors that regulate periclinal cell divisions in the moss *Physcomitrella patens*.

Dr. Ken Kosetsu and his colleagues found that one GRAS transcription factor is required for periclinal cell divisions, while another is required for anticlinal cell divisions. We identified that the former GRAS transcription factor represses the expression of the latter GRAS transcription factor. This regulation seems to determine the location where the division axis is changed from an anticlinal to a periclinal manner

Regulation of local cell growth underlies the geometric shape formation of individual cells. Cell shape is an instructive factor in oriented cell division, which guides morphogenesis in land plants. Mr. Liechi Zhang, a graduate student in our lab, found that a transporter mutant, which belongs to the *ABC* gene family, exhibited a cell shape abnormality. We are presently investigating its underlying mechanism.

This is a collaborative project that is being undertaken by our division and Dr. Takashi Murata (Kanagawa Institute of Technology) and Dr. Rumiko Kofuji (Kanazawa University), Dr. Hiroyuki Sekimoto (Japan Women's University), and Atsushi Mochizuki (RIKEN).

III. DNA damage triggers reprogramming of differentiated cells into stem cells with STEMIN

Plants are better able to undergo cellular reprogramming from differentiated cells to stem cells compared to animals. The genetic regulatory networks of reprogramming induced by phytohormones and wounding accompanying cell death have been extensively studied. We previously investigated the molecular mechanisms underlying reprogramming caused by wounding in the moss Physcomitrella patens. This year, we reported the unexpected identification of a new genetic regulatory pathway of reprogramming induced by moderate, repairable DNA damage in the absence of exogenous phytohormone treatment and wounding. When intact leafy shoots were transiently exposed to DNA damage-inducing reagents, the DNA damage was successfully repaired and the leaf cells changed to stem cells, which subsequently produced leafy shoots. Furthermore, we found that this pathway depends on the AP2/ERF transcription factor STEMIN, whose ectopic expression changes intact differentiated leaf cells into stem cells (Ishikawa et al. 2019 Nat. Plants 5: 681). Consequently, this response could be quite helpful to plants in nature. Since plants cannot immediately escape from severe environments that induce DNA damage, the conversion of differentiated cells into stem cells, which proliferate and produce new shoots, allows plants to escape from harsh environments to grow in safer places. Our findings shed light on an unforeseen pathway from the induction of DNA breaks to cellular reprogramming. An international joint graduate student Ms. Nan Gu from Dr. Chunli Chen's laboratory in the Huazhong Agricultural University mainly worked on this project (Gu et al. 2000. Nat. Plants).



Figure 1. The DNA damage reagent zeocin induces reprogramming of leaf cells into stem cells.

IV. Evolution of Carnivory in Flowering Plants

Carnivorous plants attract, capture, digest, absorb, and nourish small animals with specialized leaves, so they can grow in nutrient poor environments where they can gain prominence over other plants. To better understand the evolution of botanical carnivory, we and a group of international collaborators sequenced draft genomes of the Venus flytrap, Dionaea muscipula, the waterwheel plant, Aldrovanda vesiculosa, and the sundew Drosera spatulata. Mr. Gergo Plafalvi, a graduate student and colleagues compared the genomes and identified an early whole genome duplication in the family as a source for carnivory-associated genes. In particular, the recruitment of genes to the trap from the root was a major mechanism in the evolution of carnivory, supported by family-specific duplications. These genomes belong to the gene poorest land plants sequenced thus far, suggesting a reduction of selective pressure on different processes, including non-carnivorous nutrient acquisition (Plafavi et al. 2020 Curr. Biol.).

To investigate the molecular bases of carnivory, Dr. Hideki Narukawa performed mostly comparative analysis of carnivorous pitcher leaves and non-carnivorous flat leaves in the carnivorous plant *Cephalotus follicularis*. We found that hollow formation, which was the first step in pitcher leaf development, was initiated by growth inhibition on the adaxial side of leaf primordia. This process may be regulated by the phytohormone cytokinin.

V. Calcium ions mediate memory in the Venus flytrap

The leaves of the carnivorous plant Venus flytrap, Dionaea muscipula (Dionaea) close rapidly to capture insect prey. The closure response usually requires two successive mechanical stimuli applied to the sensory hairs on the leaf blade within a span of approximately 30 seconds. An unknown biological system in Dionaea is thought to memorize the first stimulus and transduce the signal from the sensory hair to the leaf blade. A graduate student Hiraku Suda and his colleagues linked signal memory to calcium dynamics using transgenic Dionaea expressing a Ca2+ sensor. Stimulation of the sensory hair caused an increase in cytosolic Ca2+ concentration ([Ca²⁺]_{cvt}) starting in the sensory hair and spreading to the leaf blade. A second stimulus increased [Ca2+]_{cut} to an even higher level, meeting a threshold that is correlated to the leaf blade closure. Because $[Ca^{2+}]_{cyt}$ gradually decreased after the first stimulus, the $[Ca^{2+}]_{cyt}$ increase induced by the second stimulus was insufficient to meet the putative threshold for movement after about 30 seconds. The Ca²⁺ wave triggered by mechanical stimulation moved an order of magnitude faster than that induced by wounding in petioles of Arabidopsis thaliana and Dionaea. The capacity for rapid movement has evolved repeatedly in flowering plants. This study opens a path to investigate the role of Ca²⁺ in plant movement mechanisms and their evolution.



Figure 2. Fluorescence images of a GCaMP6f Dionaea leaf blade after a sensory hair was stimulated with a needle.

VI. Molecular mechanisms and evolution of the generation and transmission of action potential in plants

Plants lack blood flow and nerves, but have evolved unique long-distance intercellular signaling mechanisms. Signals using plant hormones, peptides, proteins, and slow calcium waves have been well elucidated; however, the molecular mechanism of long-range, rapid, intercellular signaling by action potentials with fast calcium waves, which evolved in parallel to similar signaling mechanisms in animals, remains largely unknown. Rapid transmission of action potentials has been reported in specific tissues of the sensitive plant Mimosa pudica, the Venus flytrap Dionaea muscipula, the wheel plant Aldrovanda vesiculosa, and the sundew Drosera rotundifolia. We use these four species, as well as Arabidopsis thaliana, to study the molecular mechanisms of action potential generation and transmission. We have obtained genome sequences for these species and established techniques for transformation. We intend to screen for genetic factors responsible for fast intracellular and intercellular electrical signaling by action potentials, analyze their molecular characteristics such as effects on ion permeability and intracellular localization, and perform genetic gain- and loss-of-function experiments. This will allow us to understand the general mechanisms of action potential transmission in plants and the evolutionary process that resulted in diversity of transmission velocity, which is adaptive in the three plants.

Publication List:

[Original papers]

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[Review article]

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