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, 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 comprise 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 cell division axis as a grand plan of plant developmental evolution

The cell division axis has to 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*. In addition to identifying these factors, we also intend to study the spatiotemporal regulatory mechanisms will in order to understand the basis of body plan evolution in comparison to those found in the flowering plant *Arabidopsis thaliana* and the green algae *Closterium peracerosum–strigosum-littorale*.

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 decide the location where the division axis is changed from anticlinal to periclinal manner

From the observation of microtubule dynamics using the GFP-a-tubulin as a marker in the presence or absence of the GRAS transcription factor, Dr. Kosetsu's team found that the progression of the cell cycle was regulated by the GRAS transcription factor. This result suggests that the division axis is determined through the cell cycle-dependent cell shape.

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 found that a transporter mutant, which belongs to the *ABC* gene family, exhibited a cell shape abnormality. This led to a retarded gametophore development of *Physcomitrella patens*. Time-lapse imaging of the fluorescent protein tagged transporter revealed a positive correlation between the membrane localization of this transporter and local cell growth. We are presently investigating the underlying mechanism using knock-out, knock-in, and inducible overexpression transgenic plants in wild type and cytoskeleton marker lines of *P. patens*.

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

III. Evolution of Elaborated Cell Division Machinery: Spindle body

During mitosis, all eukaryotic cells divide chromosomes into two daughter cells using a bipolar mitotic spindle, which is composed of microtubules. The centrosomes, which act as microtubule organizing centers, induce formation of the two poles in metazoan cells. In contrast, the land plants cells and their sister group, zygnematales green algae, form a bipolar spindle in the absence of centrosomes. In order to understand the mechanism of acentrosomal spindle formation, the steps of microtubule reorganization during spindle formation should be visualized. We collaborated with Prof. Tomomi Nemoto of Hokkaido University and developed a two-

photon spinning disk confocal microscope, which enables 3-dimensional imaging of living cells with high temporal and spatial resolution. We also established a minispindle system, which involves a bipolar microtubule complex composed of an isolated chromosome and microtubules in tobacco cells. Analyses of microtubule behavior in the minispindle are underway in collaboration with Dr. Daisuke Tamaoki (Toyama Univ.) with Takashi Murata being the coordinating researcher for this study.

IV. Evolution of Regeneration: Genetic Regulatory Networks of Reprogramming of Differentiated Cells to Stem Cells

Both land plants and metazoa have the capacity to reprogram differentiated cells to stem cells. In the moss *Physcomitrella patens*, the leaf excision induces the reprogramming of differentiated leaf cells next to the excision to stem cells. We found that histone H3.3 chaperone HIRA proteins are induced in the leaf cells next to the excision, and positively regulate reprogramming. The role of HIRAs in reprogramming partly depends on the plant-specific transcription factor genes *SQUAMOSA PROMOTER BINDING PROTEINs* (*PpSBPs*). *PpSBPs* are involved in the repression of the reprogramming and HIRAs are necessary for the repression of *PpSBPs* through histone modifications during the reprogramming. Characterization of *HIRA*s and *PpSBPs* are in progress and are mainly being conducted by Yukiko Kabeya and Yosuke Tamada.

V. Evolution of Regeneration: Master Regulator for Reprogramming *STEMIN*

Epigenetic modifications, including histone modifications, stabilize cell-specific gene expression programs to maintain cell identities in both metazoans and land plants. Notwithstanding the existence of these stable cell states, in land plants, stem cells are formed from differentiated cells during post-embryonic development and regeneration, indicating that land plants have an intrinsic ability to regulate epigenetic memory to initiate a new gene regulatory network. However, it is less well understood how epigenetic modifications are locally regulated to influence specific genes necessary for cellular changes without affecting other genes in a genome. In this study, we found that ectopic induction of the AP2/ERF transcription factor STEMIN1 in leaf cells of the moss *Physcomitrella patens* decreases a repressive chromatin mark, histone H3 lysine 27 trimethylation (H3K27me3), on its direct target genes before cell division, resulting in the conversion of leaf cells to chloronema apical stem cells. STEMIN1 and its homologs positively regulate the formation of secondary chloronema apical stem cells from chloronema cells during development. Our results suggest that STEMIN1 functions in an intrinsic mechanism underlying local H3K27me3 reprogramming to initiate stem cell formation. Masaki Ishikawa is this study's coordinating researcher.

We also found that a component of the DNA repair machinery functions in the STEMIN1-induced stem cell formation in leaves. This study is mainly being conducted by Ruan de Villiers.

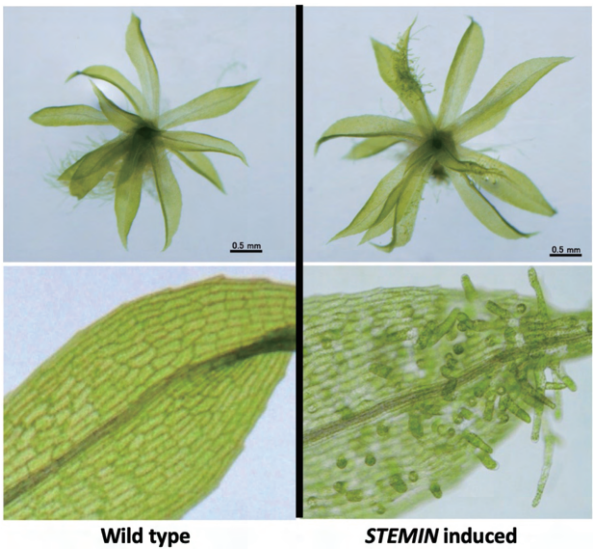

Figure 1. STEMIN can change leaf cells into stem cells.

VI. Evolution of Regeneration: Other pathways

Nan Gu, a joint graduate student at both Huazhong Agricultural University and NIBB whose research interest is the relationship between DNA damage and the reprogramming from differentiated cells to stem cells, has found that DNA damage is a novel trigger to induce the reprogramming without wounding or dead cells in *P. patens*.

VII. Evolution of Molecular Mechanisms of Plant Movement

The sensitive plant *Mimosa pudica* and the Venus fly trap *Dionaea muscipula* have long attracted the interest of researchers due to their spectacular leaf movements in response to touch or other external stimuli. Although various aspects of these movements have been elucidated by physiological approaches, the lack of genetic tools available has hampered the investigation of molecular mechanisms involved in these processes. To overcome this obstacle, we developed genetic transformation methods for these plants. Functional analysis of motor organ-enriched genes by CRISPR/Cas9-mediated knockout identified two channel genes and a transcription factor that play various roles in rapid leaf movements in *M. pudica*. We also generated a transgenic *D. muscipula* expressing a calcium sensor protein, which enables us to study how this plant counts the number of mechanical stimuli for its trap closure. The studies on *M. pudica* and *D. muscipula* were conducted mainly by Hiroaki Mano and Hiraku Suda, respectively.

VIII. Establishment of a new single cell transcriptome method

Next-generation sequencing technologies have made it possible to carry out transcriptome analysis at the single-cell level. Single-cell RNA-sequencing (scRNA-seq) data provide insights into cellular dynamics, including intercellular heterogeneity as well as inter- and intra-cellular fluctuations in gene expression that cannot be studied using populations of cells. The utilization of scRNA-seq is, however,

restricted to cell types that can be isolated from their original tissues, and it can be difficult to obtain precise positional information for these cells in situ. Here, we established single cell-digital gene expression (1cell-DGE), a method of scRNA-seq that uses micromanipulation to extract the contents of individual living cells in intact tissue while recording their positional information. With 1cell-DGE, we could detect differentially expressed genes (DEGs) during the reprogramming of leaf cells of the moss Physcomitrella patens, identifying 6382 DEGs between cells at 0 and 24 h after excision. Furthermore, we identified a subpopulation of reprogramming cells based on their pseudotimes, which were calculated using transcriptome profiles at 24 h. 1cell- DGE with microcapillary manipulation can be used to analyze the gene expression of individual cells without detaching them from their tightly associated tissues, enabling us to retain positional information and investigate cell–cell interactions.

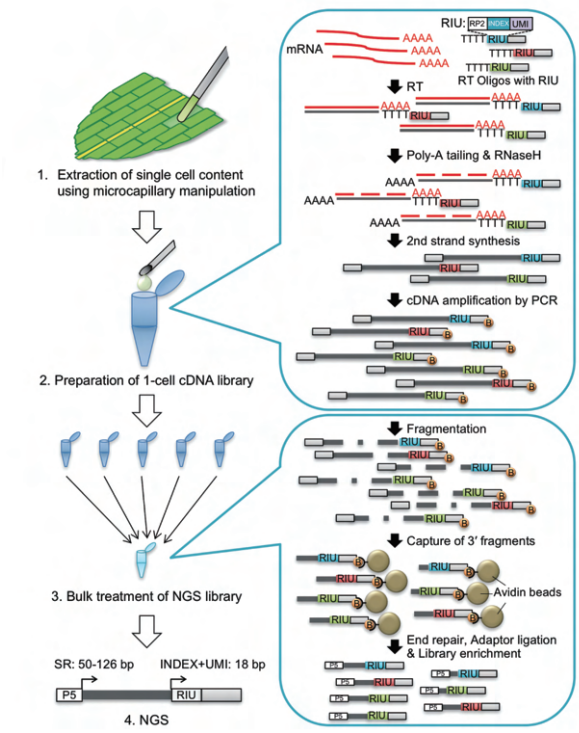

Figure 2. Schematic representation of the workflow of single cell – digital gene expression (DGE)

IX. Evolution of Carnivory in Flowering Plants

Carnivorous plants exploit animals as a source of nutrition and have inspired long-standing questions about the origin and evolution of carnivory-related traits. 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.

In order to elucidate the origin of the pitcher shape, Gergo Palfalvi, a graduate student, looked into the initiation factors separating the flat leaf and pitcher leaf establishment in the primordia. Mass sequencing of shoot apices among several environmental conditions utilized to alter the leaf/pitcher ratio are in progress. We are also working on refinement of the genome especially in epigenetic studies.

Publication List:

〔Original papers〕

- Cai, H., Li, Q., Fang, X., Li, J., Curtis, N.E., Altenburger, A., Shibata, T., Feng, M., Maeda, T., Schwartz, J.A., Shigenobu, S., Lundholm, N., Nishiyama, T., Yang, H., Hasebe, M., Li, S., Pierce, S.K., and Wang, J. (2019). Data descriptor: A draft genome assembly of the solar-powered sea slug *Elysia chlorotica*. Sci. Data *6*, 190022. doi: 10.1038/sdata.2019.22
- Hashida, Y., Takechi, K., Abiru, T., Yabe, N., Nagase, H., Hattori, K., Takio, S., Sato, Y., Hasebe, M., Tsukaya, H., and Takano, H. (2020). Two ANGUSTIFOLIA genes regulate gametophore and sporophyte development in Physcomitrella patens. Plant J. *101*, 1318-1330. doi: 10.1111/tpj.14592
- Kakishima, S., Liang, Y.-S., Ito, T., Yang, T.-Y.A., Lu, P.-L., Okuyama, Y., Hasebe, M., Murata, J., and Yoshimura, J. (2019). Evolutionary origin of a periodical mass-flowering plant. Ecol. Evol. *9*, 4373-4381. doi: 10.1002/ece3.4881
- Ishikawa, M., Morishita, M., Higuchi, Y., Ichikawa, S., Ishikawa, T., Nishiyama, T., Kabeya, Y., Hiwatashi, Y., Kurata, T., Kubo, M., Shigenobu, S., Tamada, Y., Sato, Y., and Hasebe, M. (2019). Physcomitrella STEMIN transcription factor induces stem cell formation with epigenetic reprogramming. Nat. Plants *5*, 681-690. doi: 10.1038/s41477-019-0464-2
- Kubo, M., Nishiyama, T., Tamada, Y., Sano, R., Ishikawa, M., Murata, T., Imai, A., Lang, D., Demura, T., Reski, R., and Hasebe, M. (2019). Single-cell transcriptome analysis of Physcomitrella leaf cells during reprogramming using microcapillary manipulation. Nucleic Acids Res. *47*, 4539-4553. doi: 10.1093/nar/gkz181
- Kumar, M., Quan, X., Awatsuji, Y., Cheng, C., Hasebe, M., Tamada, Y., and Matoba, O. (2020). Common-path multimodal three-dimensional fluorescence and phase imaging system. J. Biomed. Opt. *25*, 1-15. doi: 10.1117/1.JBO.25.3.032010
- Sasaki, T., Tsutsumi, M., Otomo, K., Murata, T., Yagi, N., Nakamura, M., Nemoto, T., Hasebe, M., and Oda, Y. (2019). A Novel Katanin-Tethering Machinery Accelerates Cytokinesis. Current Biol. *29*, 4060-4070. doi: 10.1016/j.cub.2019.09.049

〔Review article〕

- Renner, T., Lan, T., Farr, K.M., Ibarra-Laclette, E., Herrera-Estrella, L., Schuster, S.C., Hasebe, M., Fukushima, K., and Albert, V.A. (2018). Carnivorous plant genomes. In: Carnivorous Plants: Physiology, Ecology, and Evolution, A.M. Ellison and L. Adamec, eds. (Oxford University Press), pp. 135-153.