DIVISION OF EVOLUTIONARY BIOLOGY

Professor
HASEBE, Mitsuyasu

Associate Professor
MURATA, Takashi

Assistant Professor:	*TAMADA, Yosuke*
	ISHIKAWA, Masaki
Technical Staff:	*KABEYA, Yukiko*
NIBB Research Fellow:	*IMAI, Akihiro*
Postdoctoral Fellow:	*MANO, Hiroaki*
	TORIBA, Taiyo
SOKENDAI Graduate Student:	*KAMIDA, Chiharu*
	KOSHIMIZU, Shizuka
	LI, Chen
	MORISHITA, Mio
	PALFALVI, Gergo
	SUDA, Hiraku
	SUGAYA, Tomomi
	ZHANG, Liechi
	HORIUCHI, Yuta
	DE VILLIERS, Ruan
Visiting Graduate Student:	*GU, Nan*
Visiting Undergraduate:	*SAKAZAKI, Takuya*
Technical Assistant:	*AOKI, Etsuko*
	GOTO, Misako
	HIRAMATSU, Mika
	KAJIKAWA, Ikumi
	MORI, Asuka
	MATSUZAKI, Yoko
	NISHI, Tayo
	OOI, Shoko
Secretary:	*KOJIMA, Yoko*

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, there still remain unexplained phenomena, one of which is the evolution of complexity. It is difficult to explain the mechanisms needed to evolve complex adaptive traits at cellular and organismal levels, such as cell division machinery, regeneration, novel organ development, host race change, and mimicry. Such traits comprise many components and become adaptive only when all components 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 infer the mechanisms needed to evolve complex characters.

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

Cell division axis has to be properly regulated during development in both metazoans and land plants. Genetic changes in the regulation of cell division axis lead to the development of multicellular organisms. Since land plants do not have centrosomes and asteroid bodies, both of which are involved in the axis formation of metazoans, land plants should 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 identify the factors, the spatiotemporal regulatory mechanisms will be studies to understand the basis of body plan evolution with comparison to those in the flowering plant *Arabidopsis thaliana* and the green algae *Closterium peracerosum–strigosum-littorale*. This is a collabortion project between our division and Dr. Rumiko Kofuji in Kanazawa University, Dr. Hiroyuki Sekimoto in Japan Women's University, and Atsushi Mochizuki in RIKEN.

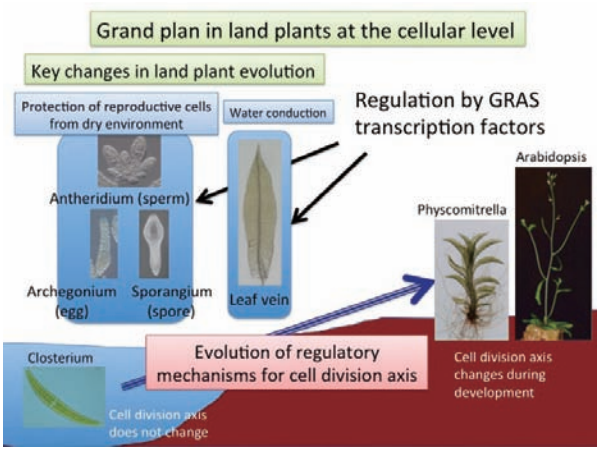

Figure 1. Evolution of the regulatory mechanisms for cell division axis appears to be the basic change leading to the subsequent divergence of land plants.

III. Evolution of Elaborated Cell Division Machinery: Spindle body

At mitosis, all eukaryotic cells divide chromosomes to 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 cells of land plants and their sister group, zygnematales green algae, form the bipolar spindle in the absence of centrosomes. For understanding the mechanism of acentrosomal spindle formation, the steps of microtubule reorganization during spindle formation should be visualized. We collaborated with Prof. Tomomi Nemoto in 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 found that spindle microtubules elongate from a prospindle, that is, a microtubule cage with two poles on the nuclear envelope. Our data suggest that the prospindle organizes the bipolar spindle, as centrosomes do in metazoan cells. In contrast to the metazoan centrosomes, however, the prospindle disappears before metaphase. To understand the mechanism how the bipolar spindle is maintained in the absence of the organizer, we 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 in progress. Takashi Murata is a main researcher of this study.

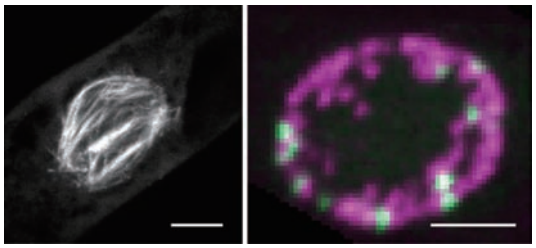

Figure 2. Microtubules (left) and chromosomes (right) during spindle formation of tobacco BY-2 cells. In the right panel, Chromosomes and centromeres are shown as magenta and green, respectively. Bar, 10 μm.

IV. Evolution of Regeneration: Reprogramming of Differentiated Cells to Pluripotent Stem Cells

Different species have different morphology and also cellular characters vary between species. Stem cells self-renew and repeatedly produce differentiated cells during development. Conversely, differentiated cells can be converted into stem cells in some organisms. In plants, the reprogramming to a stem cell can lead to generation of a new individual, which is an effective strategy for propagation. The ability to reprogram is different from species to species but the reason is unknown. The moss *Physcomitrella patens* has a rapid reprogramming ability and is feasible for use in experiments. Cells in a dissected leaf are reprogrammed to become chloronema apical stem cells within 24 hours. We found that *P. patens COLD SHOCK DOMAIN PROTEIN* genes (*PpCSP*s) are positive regulators of the reprogramming in Physcomitrella. Stem-cell formation was enhanced in the over-expression lines of a *PpCSP* gene (Figure 3). Quadruple deletion mutants of *PpCSPs* exhibited attenuated reprogramming. PpCSPs share conserved domains with an induced pluripotent stem (iPS) cell factor Lin28 in mammals, indicating that closely related proteins function in the enhancement of reprogramming in both land plant and metazoan lineages. This research is mainly performed by Chen Li and Yosuke Tamada.

The stem cell formation requires light and wounding signals. We found that the stem cell formation was facilitated in the quadruple deletion mutant of the *P. patens SQUAMOSA promoter binding protein* (*PpSBP*) genes, some of which are known to be repressed by light signals. In addition, we found that *PpSBPs* are negatively regulated by the wounding signal. Characterization of *PpSBPs* are in progress mainly by Yukiko Kabeya and Yosuke Tamada to investigate cross talk between light and wounding signaling pathways in the process of stem cell formation.

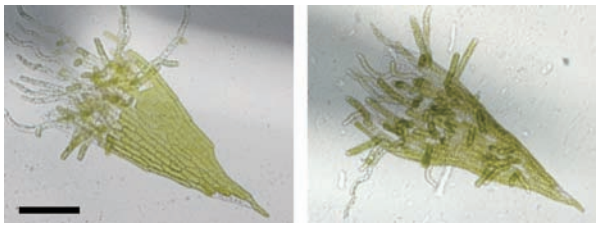

Figure 3. Leaves of wild type (left) and *PpCSP1* Bar, 200 μm.

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

Animal somatic cells can be reprogrammed to iPS cells by introducing four transcription factors, while such factors have not been identified in plants. We have previously identified a gene encoding a member of a plant-specific transcription factor, STEM CELL-INDUCING FACTOR 1 (STEMIN1) that was able to induce direct reprogramming of differentiated leaf cells into chloronema apical stem cells without wounding signals. *STEMIN1* and its two paralogous genes (*STEMIN2* and *STEMIN3*) were activated in leaf cells that underwent reprogramming. In addition, deletion of the three *STEMIN* genes delayed reprogramming after leaf excision, suggesting that these genes redundantly function in the reprogramming of cut leaves. We next examined whether the three *STEMIN* genes also function in stem cell formation in regular protonemal development, formation of chloronema side branch initial cells. We detected promoter activities of *STEMIN1* and its paralogs in chloronema cells undergoing side branch formation (Figure 4). On the other hand, the activities were not detected in chloronema apical stem cells. In addition, the frequency of side branch formation in the triple deletion mutant was significantly lower than that in the wild type. These results suggest that STEMIN1 and its paralogs participate in the formation of chloronema side branch initial cells, but not in the maintenance of chloronema apical stem cells. To understand the role of STEMIN1 in reprogramming, we investigate STEMIN1-direct target genes identified by RNA-seq and ChIP-seq analyses. Masaki Ishikawa and Mio Morishita are this study's main researchers.

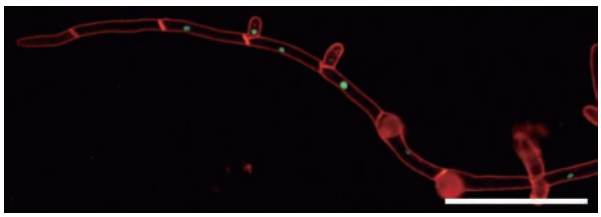

Figure 4. Promoter activity of the STEMIN1 gene in protonemata. Green and red represent the promoter activity and plasma membrane stained with FM4-64, respectively. Scale bar, 100 μm.

VI. Evolution of Regeneration: Stem cells laterally inhibit surrounding cells

Singly isolated leaf cells are reprogramed into stem cells in *P. patens*. However, only one cell of two longitudinally isolated adjacent cells becomes a stem cell and the other appears to be laterally inhibited by the cell to be a future stem cell. The fourth-year graduate student Liechi Zhang is investigating the factors involved in the lateral inhibition.

VII. Evolution of Regeneration: Other pathways

Nan Gu, a fifth-year joint graduate student between Huazon Agricultural University and NIBB is interested in DNA damage and reprogramming, and is working with the mechanisms connecting DNA damage and reprogramming of differentiated cells to stem cells.

We found that INHIBITOR OF GROWTH (ING) proteins are involved in the stem cell formation of cut leaves. The ING proteins are known to regulate an apoptosis pathway in animals but plants do not have the corresponding pathway. Akihiro Imai, a former postdoc in this division and now an Assistant Professor in Hiroshima Institute of Technology is investigating the molecular function of ING as a collaboration work.

VIII. Molecular mechanisms of Plant Movement using *Mimosa pudica*

The sensitive plant *Mimosa pudica* has long attracted the interest of researchers due to its spectacular leaf movements in response to touch or other external stimuli. Although various aspects of the seismonastic movement have been elucidated by physiological, cytological or biochemical approaches, the lack of genetic tools hampered the investigation of molecular mechanisms involved in these processes. To overcome this obstacle, we sequenced and analyzed the genome in a collaborative project with Dr. Chao-Li Huang in National Cheng Kung University. Furthermore, we developed an efficient genetic transformation method for *M. pudica* (Mano *et al*., 2014) and established a CRISPR/Cas9-mediated gene knock-out system. This year we isolated several candidate genes that may play roles in the seismonastic movement by comparing gene expression profiles between motor organs (pulvini) and non-motor organs and between extensor and flexor halves of pulvini. Functional analyses of these genes with the CRISPR/Cas9 system are in progress.

In addition to the reverse genetic analyses, we have challenged whole-organ 3D imaging of tertiary pulvini to comprehensively understand the volume and shape changes of individual cells during movements. To this end, we extensively optimized fixation, staining and tissue clearing methods and succeeded in obtaining images that covered the entire region of a tertiary pulvinus with sufficient resolution. We have also been developing computational methods to automatically extract and analyze the volumes and shapes of thousands of cells in a pulvinus. This study was conducted mainly by Hiroaki Mano.

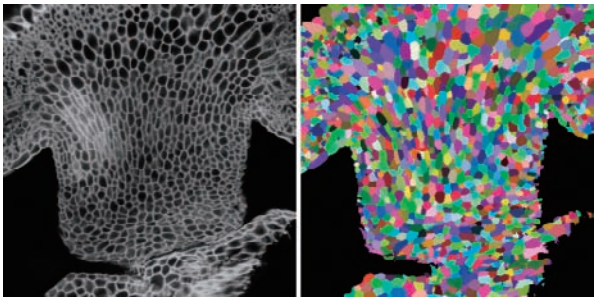

Figure 5. Quantitative 3D image analysis of a tertiary pulvinus of *Mimosa pudica* at the whole organ level.
Left: Cell walls stained by a newly developed method.
Right: Automatic segmentation of the individual cells

IX. Evolution of plant development

To investigate evolution of novel complex traits, the following studies are ongoing with graduate students: The fifth-year graduate student Chiharu Kamida studies genes involved in movable tentacle development in the sandew *Drosera spatulata*. The fourth-year graduate student Shizuka Koshimizu is interested in the evolution of floral homeotic genes and investigates the function of MADS-box genes in the non-flowering plant *Physcomitrella patens*. The pseudanthium is a flower-like inflorescence, the molecular mechanisms of the development of which are unknown. The fifth-year graduate student Tomomi Sugaya succeeded in transferring the *FT* gene from *Arabidopsis thaliana* into the pseudanthium *Houttuynia cordata*. Furthermore, introduction of the *FT* gene successfully induced flowers.

Publication List:

〔Original papers〕

- Tong, W., Imai, A., Tabata, R., Shigenobu, S., Yamaguchi, K., Yamada, M., Hasebe, M., Sawa, S., Motose, H., and Takahashi, T. (2016). Polyamine resistance is increased by mutations in a nitrate transporter gene NRT1.3 (AtNPF6.4) in *Arabidopsis thaliana*. Front. Plant Sci. *7*, 1–22.
- Pereman, I., Mosquna, A., Katz, A., Wiedemann, G., Lang, D., Decker, E.L., Tamada, Y., Ishikawa, T., Nishiyama, T., Hasebe, M., et al. (2016). The Polycomb group protein CLF emerges as a specific tri-methylase of H3K27 regulating gene expression and development in *Physcomitrella patens*. Biochim. Biophys. Acta *1859*, 860–870.
- Plavskin, Y., Nagashima, A., Perroud, P.-F., Hasebe, M., Quatrano, R.S., Atwal, G.S., and Timmermans, M.C.P. (2016). Ancient trans-acting siRNAs confer robustness and sensitivity onto the auxin response. Dev. Cell *36*, 276-289.
- Nagata, T., Hasebe, M., Toriba, T., Taneda, H., and Crane, P.R. (2016). Sex conversion in *Ginkgo biloba* (Ginkgoaceae). J. Jap. Bot. *91 Suppl*., 120-127.

〔Review article〕

- Hasebe, M. (2016). Starting bell for embryos. Nat. Plants *2*, 1-2.