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*
	SHIBATA, Tomoko
	TAMAOKI, Daisuke
	TORIBA, Taiyo
SOKENDAI Graduate Student:	*FUKUSHIMA, Kenji*
	KAMIDA, Chiharu
	KOSHIMIZU, Shizuka
	LI, Chen
	MORISHITA, Mio
	PALFALVI, Gergo
	SUDA, Hiraku
	SUGAYA, Tomomi
	ZHANG, Liechi
	HORIUCHI, Yuta
Visiting Graduate Student:	*GU, Nan*
Visiting Scientist:	*BISOVA, Katerina*
	ČUPEROVÁ, Zuzana
	KAWASHIMA, Takeshi
	LI, Liu
	LI, Ping
	TUROCZY, Zoltan
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. 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, regeneration of a stem cell can lead to generation of a new individual, which is an effective strategy for propagation. The ability in reprogramming 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), homologous to mammal induced pluripotent stem (iPS) cell factor LIN28, are positive regulators of the stem-cell formation in Physcomitrella. Characterization of *PpCSP*s are ongoing by Chen Li and Yosuke Tamada.

We also found that the stem cell formation required light signaling mediated by red and blue light receptors. In addition, 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 the blue-light receptor CRYPTOCHROME. In addition, the histone chaperone genes, *Histone gene repressor A* (*HIRA*) were found to regulate chromatin modification of *PpSBP*s. Characterization of these genes are in progress mainly by Yukiko Kabeya to investigate the cross talk between light and chromatin modification pathways in the process of the stem cell formation.

III. 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 also found that three *STEMIN* genes function redundantly in chloronema apical stem cell formation from chloronema cells during chloronema development. On the other hand, in contrast to *STEMIN1*, induction of *STEMIN2* or *STEMIN3* in gametophores and chloronemata did not induce formation of chloronema apical stem cells. These results indicate that *STEMIN1* has sufficient ability to change intact leaf cells to stem cells, but its paralogous genes do not. Masaki Ishikawa and Mio Morishita were this study's main researchers.

IV. Evolution of Regeneration: Lateral inhibition by stem cells to surrounding cells to be stem 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. Liechi Zhang is investigating the factors involved in the lateral inhibition.

V. Evolution of Regeneration: Other pathways

Nan Gu 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 is mainly investigating the molecular function of ING.

VI. Cell Cycle Reentry from the Late S Phase in Physcomitrella

At mitosis, all eukaryotic cells divide chromosomes to two daughter cells using a mitotic spindle, which is composed of microtubules. Differentiated cells are in a non-dividing, quiescent state, but some differentiated cells can reenter the cell cycle in response to appropriate stimuli. Quiescent cells are generally arrested at the G0/G1 phase, reenter the cell cycle, and progress to the S phase to replicate their genomic DNA. On the other hand, some types of cells are arrested at different phases and reenter the cell cycle from there. In the moss *Physcomitrella patens*, the differentiated leaf cells of gametophores formed in the haploid generation contain approximately 2C DNA content, and DNA synthesis is necessary for reentry into the cell cycle, which is suggested to be arrested at late S phase. Masaki Ishikawa reviewed various cell-division reactivation processes in which cells reenter the cell cycle from the late S phase, and discussed possible mechanisms of such unusual cell cycle reentries with special emphasis on Physcomitrella (Ishikawa and Hasebe 2015).

VII. 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 suggests 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. Daisuke Tamaoki and Takashi Murata were this study's main researchers.

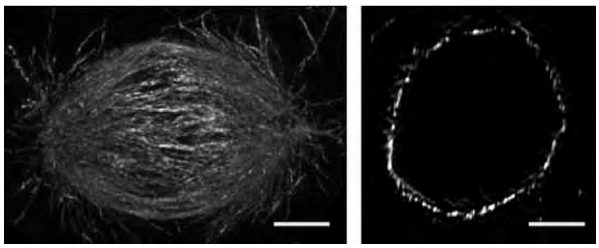

Figure 1. Prospindle of a tobacco BY-2 cell. Left: projection image, Right: cross section image of the same cell. Bar, 5 μm.

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 approaches, the lack of genetic tools has hampered the investigation of molecular mechanisms involved in these processes. To overcome this obstacle, we developed an efficient genetic transformation method for *M. pudica* (Mano *et al*., 2014) and then established a CRISPR/Cas9-mediated gene knock-out system. By using these new techniques, we succeeded in generating immotile *M. pudica* mutants. Further characterization of these mutants will provide insights into the mechanism of leaf movements as well as their adaptive meanings. Another promising approach based on the transgenic technique is molecular imaging using fluorescent reporter proteins. We generated transgenic plants expressing fluorescent proteins which can visualize actin cytoskeleton and calcium ions, respectively. We are now attempting live-imaging of these factors, which participate in the seismonastic movement, but their roles still remain unclear. This study was conducted mainly by Hiroaki Mano.

IX. Molecular mechanisms of mimicry

An excellent example of mimicry is the flower-mimicry of the orchid mantis *Hymenopus coronatus* with pink and white coloration and petal-like legs. Biochemical analyses indicated that the reduced form of xanthommatin, a common red pigment of the ommochrome family, almost solely contributes to the pink color. We also analyzed the ultrastructure of pigment granules by transmission electron microscopy. We found that integumentary cells of *H. coronatus* contain a numerous number of electron-dense granules with 60-nm diameters. This result suggests that this special intracellular structure may contribute to the unique pink color of *H. coronatus*. This work was mainly done by Hiroaki Mano.

X. Oriented cell division shapes carnivorous pitcher leaves of *Sarracenia purpurea*

Complex morphology is one of the major evolutionary outcomes of phenotypic diversification. In some carnivorous plants, the planar leaves of common ancestors have been modified to form a pitcher shape. However, how leaf development was altered in the evolution of pitcher leaves remains unknown. Here we show that the pitcher leaves of *Sarracenia purpurea* develop through cell division patterns of adaxial tissues that are distinct from those in bifacial and peltate leaves, subsequent to regular expression of adaxial and abaxial marker genes. Differences in the orientation of cell divisions in the adaxial domain cause bifacial growth in the distal region and adaxial ridge protrusion in the middle region. These different growth patterns in a leaf establish the pitcher morphology. A computer simulation suggests that the cell division plane is critical for the acquisition of the novel pitcher morphology. Our results imply that tissue-specific changes in the orientation of cell division underlie the development of a morphologically complex leaf (Fukushima et al. 2015).

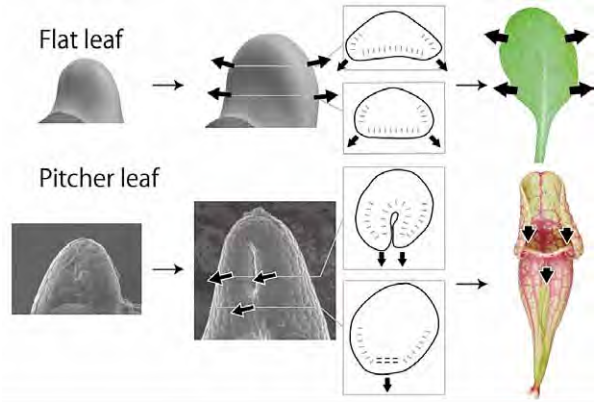

Figure 2. Schematics of leaf development in flat leaf and pitcher leaf. Growth directions are indicated by arrows. Lines show cell division planes.

To further investigate the evolution of pitcher leaves, we selected the Australian pitcher plant *Cephalotus follicularis*, which produces both carnivorous pitcher and non-carnivorous flat leaves, enabling us to deduce carnivory-related genes by comparative approaches in a single species. To understand the genomic changes associated with the evolution of carnivory, we sequenced 2-Gbp genome of *C. follicularis*. Whole-genome shotgun data corresponding to 100x coverage were produced by Illumina paired-end/mate-pair sequencing with 180-bp to 5-kb insert sizes, and *de novo* assembly yielded 15.6 kb of contig N50 and 78 kb of scaffold N50. 14 Gbp of PacBio reads with 2-kb mean max subread length were produced for gap filling. Transcript-based gene prediction with RNA-seq reads found 45,469 gene models. This study was mainly conducted by Kenji Fukushima.

XI. Evolution of plant development

To investigate evolution of novel complex traits, the following studies are ongoing: Chiharu Kamida studies genes involved in movable tentacle development in the sandew *Drosera spatulata*. Shizuka Koshimizu is interested in the evolution of floral homeotic genes and investigates the function of MADS-box genes in non-flowering plants *Physcomitrella patens*. The pseudanthium is a flower-like inflorescence, the molecular mechanisms of the development of which are unknown. 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〕

- Fukushima, K., Fujita, H., Yamaguchi, T., Kawaguchi, M., Tsukaya, H., and Hasebe, M. (2015). Oriented cell division shapes carnivorous pitcher leaves of Sarracenia purpurea. Nat. Commun. *6*, 6450.
- Kinoshita, A., ten Hove, C.A., Tabata, R., Yamada, M., Shimizu, N., Ishida, T., Yamaguchi, K., Shigenobu, S., Takebayashi, Y., Iuchi, S., *et al*. (2015). A plant U-box protein, PUB4, regulates asymmetric cell division and cell proliferation in the root meristem. Development *142*, 444-453.
- Otomo, K., Hibi, T., Murata, T., Watanabe, H., Kawakami, R., Nakayama, H., Hasebe, M., and Nemoto, T. (2015). Multi-point scanning two-photon excitation microscopy by utilizing a high-peak-power 1042-nm laser. Anal. Sci. *31*, 307-313.
- Shimizu, N., Ishida, T., Yamada, M., Shigenobu, S., Tabata, R., Kinoshita, A., Yamaguchi, K., Hasebe, M., Mitsumasu, K., and Sawa, S. (2015). BAM 1 and RECEPTOR-LIKE PROTEIN KINASE 2 constitute a signaling pathway and modulate CLE peptide-triggered growth inhibition in Arabidopsis root. New Phytol. *208*, 1104-1113.
- Teh, O.K., Hatsugai, N., Tamura, K., Fuji, K., Tabata, R., Yamaguchi, K., Shingenobu, S., Yamada, M., Hasebe, M., Sawa, S., *et al*. (2015). BEACH-domain proteins act together in a cascade to mediate vacuolar protein trafficking and disease resistance in Arabidopsis. Mol. Plant *8*, 389-398.

〔Review article〕

- Ishikawa, M., and Hasebe, M. (2015). Cell cycle reentry from the late S phase: implications from stem cell formation in the moss Physcomitrella patens. J. Plant Res. *128*, 399-419.