

## 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, 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, and novel organ development. 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 studied 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 collaboration project between our division and Dr. Rumiko Kofuji in Kanazawa University, Dr. Hiroyuki Sekimoto in Japan Women's University, and Dr. Atsushi Mochizuki in RIKEN.

## 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 also established a minispindle system, which involves a bipolar microtubule complex composed of an isolated chromosome and microtubules in tobacco cells. In collaboration with Dr. Daisuke Tamaoki (Toyama Univ.), analyses of microtubule behavior in the minispindle are in progress. Takashi Murata is a main researcher of this study.

## IV. Evolution of Regeneration: Generic 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. We found that the moss *Physcomitrella patens* Cold-Shock Domain Protein 1 (PpCSP1) regulates reprogramming of differentiated leaf cells to chloronema apical stem cells and shares conserved domains with the induced pluripotent stem cell factor Lin28 in mammals. PpCSP1 accumulates in the reprogramming cells and is maintained throughout the reprogramming process and in the resultant stem cells. Expression of *PpCSP1* is negatively regulated by its 3'-untranslated region (3'-UTR). Removal of the 3'-UTR stabilizes *PpCSP1* transcripts, results in accumulation of PpCSP1 protein, and enhances reprogramming. A quadruple deletion mutant of *PpCSP1* and three closely related *PpCSP* genes exhibits attenuated reprogramming indicating that the *PpCSP* genes function redundantly in cellular reprogramming. Taken together, these data demonstrate a positive role of PpCSP1

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (\*).

in reprogramming, which is similar to the function of mammalian Lin28 (Li et al. 2017).

We also found that histone chaperone HIRA proteins regulate stem cell formation through histone modification on *SQUAMOSA PROMOTER BINDING PROTEIN* (*PpSBP*) genes. Characterization of *HIRAs* and *PpSBPs* are in progress mainly by Yukiko Kabeya and Yosuke Tamada.

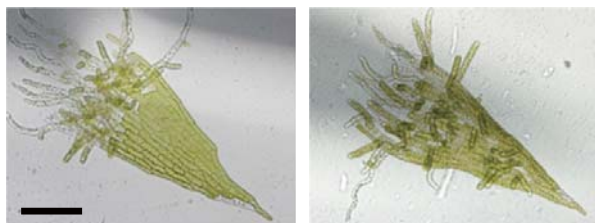


Figure 1. Cells facing the cut of dissected gametophore leaves were reprogrammed to be stem cells in wild type (left), while both edge and inner cells were reprogrammed with PpCSP1 accumulation (right).

## 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 found 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. 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.

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

Under certain circumstances differentiated cells can be reprogrammed to form stem cells in land plants, but only a portion of the cells reprogram successfully. A long-distance inhibitory signal from reprogrammed cells to surrounding cells has been reported in some ferns. We showed the existence of an anisotropic inhibitory signal to regulate stem cell formation in the moss *Physcomitrella patens*. When single cells were isolated from a gametophore leaf, over 90% of them were reprogrammed to stem cells with characteristic nuclear expansion. By contrast, when two adjacent cells were isolated, the nuclei of both cells expanded, but successful reprogramming of both cells occurred only in approximately one fifth of the pairs. When three aligned cells were isolated, the reprogramming rate of both edge cells decreased with a living middle cell but did not with a dead middle cell. Furthermore, unequal conversion into stem cells was more prominent in cell pairs aligned parallel to the proximal-distal leaf axis than in those perpendicular to the axis. This study gives an insight into the role of the inhibitory signal in development and evolution as well as efficient stem cell induction from differentiated cells (Sato et al. 2017). Liechi Zhang is investigating the factors involved in the lateral inhibition.

## VII. Evolution of Regeneration: Other pathways

Nan Gu, a joint graduate student between Huazhong 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. Evolution of Molecular Mechanisms of Plant Movement

The sensitive plant *Mimosa pudica* and the carnivorous plants sundew (*Drosera spatulata*) and 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 the 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 their genomes. Furthermore, we developed an efficient genetic transformation method for these plants and established a CRISPR/Cas9-mediated gene knock-out system in *M. pudica*. We isolated several candidate genes that may play roles in the seismonastic movement of *M. pudica* 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. The studies on *M. pudica* and carnivorous plants were conducted mainly by Hiroaki Mano and Hiraku Suda, respectively.

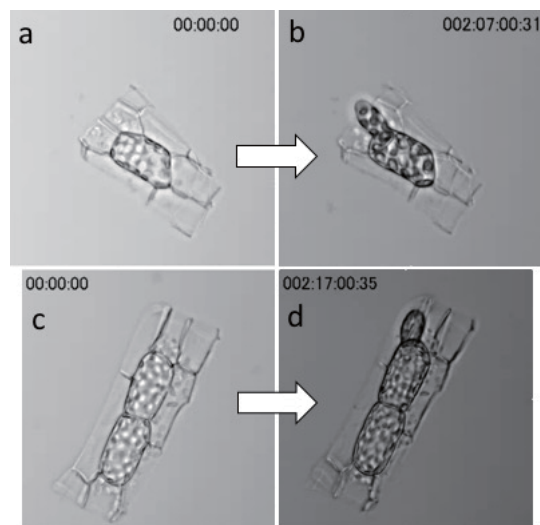


Figure 2. Reprogramming of isolated gametophore leaf cells of the moss *Physcomitrella patens*. A single cell (a, b) and two adjacent cells (c, d) were isolated by hand using a carbon knife.

## IX. Evolution of Plant Development

To investigate evolution of novel complex traits, the following studies are ongoing with graduate students: Chiharu Kamida studies genes involved in movable tentacle development in the sundew *Drosera spatulata*. 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. 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. Ruan de Villiers investigates the evolution of signaling pathways of the phytohormone strigolactone in land plants.

## X. Evolution of Digestive Enzymes and Pitcher Leaves in Carnivorous Plants

Carnivorous plants exploit animals as a nutritional source and have inspired long-standing questions about the origin and evolution of carnivory-related traits. To investigate the molecular bases of carnivory, we sequenced the genome of the heterophyllous pitcher plant *Cephalotus follicularis*, in which we succeeded in regulating the developmental switch between carnivorous pitcher and non-carnivorous flat leaves by using different temperatures. Transcriptome comparison of the two leaf types and gene repertoire analysis identified genetic changes associated with prey attraction, capture, digestion and nutrient absorption. Analysis of digestive fluid proteins from *C. follicularis* and three other carnivorous plants with independent carnivorous origins revealed repeated co-options of stress-responsive protein lineages coupled with convergent amino acid substitutions to acquire digestive physiology. These results imply constraints on the available routes to evolve plant carnivory.

To further investigate the molecular mechanisms to regulate flat and pitcher leaves, Gergo Palfalvi analyzes transcriptomes and chromatin modifications as a collaboration work with Dr. Kenji Fukushima in Colorado University.

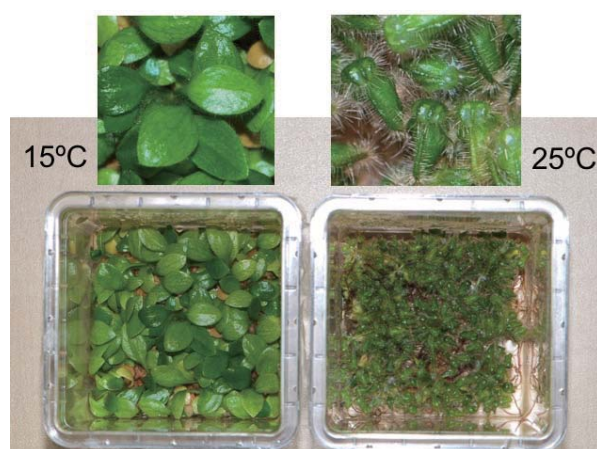


Figure 3. *Cephalotus follicularis* preferentially produces flat leaves at 15°C (left) and pitcher leaves at 25°C.

## Publication List:

### [Original papers]

- Fukushima, K., Fang, X., Alvarez-Ponce, D., Cai, H., Carretero-Paulet, L., Chen, C., Chang, T.-H., Farr, K.M., Fujita, T., Hiwatashi, Y., Hoshi, Y., Imai, T., Kasahara, M., Librado, P., Mao, L., Mori, H., Nishiyama, T., Nozawa, M., Pálfalvi, G., Pollard, S.T., Rozas, J., Sánchez-Gracia, A., Sankoff, D., Shibata, T.F., Shigenobu, S., Sumikawa, N., Uzawa, T., Xie, M., Zheng, C., Pollock, D.D., Albert, V.A., Li, S., and Hasebe, M. (2017). Genome of pitcher plant *Cephalotus* reveals genetic changes associated with carnivory. *Nat. Ecol. Evol.* 1, 0059.
- Hattori, M., Tamada, Y., Murata, T., Oya, S., Hasebe, M., Hayano, Y., and Kamei, Y. (2017). Artificial testing targets with controllable blur for adaptive optics microscopes. *Opt. Eng.* 56, 080502.
- Kosetsu, K., Murata, T., Yamada, M., Nishina, M., Boruc, J., Hasebe, M., Damme, D.V., and Goshima, G. (2017). Cytoplasmic MTOCs control spindle orientation for asymmetric cell division in plants. *Proc. Natl. Acad. Sci. USA* 114, E8847-E8854.
- Li, C., Sako, Y., Imai, A., Nishiyama, T., Thompson, K., Kubo, M., Hiwatashi, Y., Kabeya, Y., Karlson, D., Wu, S.-H., Ishikawa, M., Murata, M., Benfey, P.N., Sato, Y., Tamada, Y., and Hasebe, M. (2017). A Lin28 homolog reprograms differentiated cells to stem cells in the moss *Physcomitrella patens*. *Nat. Commun.* 8, 14242.
- Sato, Y., Sugimoto, N., Hirai, T., Imai, A., Kubo, M., Hiwatashi, Y., Nishiyama, T., and Hasebe, M. (2017). Cells reprogramming to stem cells inhibit the reprogramming of adjacent cells in the moss *Physcomitrella patens*. *Sci. Rep.* 7, 1909.
- Yilamujiang, A., Zhu, A., Ligabue-Braun, R., Bartram, S., Witte, C.P., Hedrich, R., Hasabe, M., Schöner, C.R., Schöner, M.G., Kerth, G., Carlini, C.R., and Mithöfer, A. (2017). Coprophagous features in carnivorous *Nepenthes* plants: a task for ureases. *Sci. Rep.* 7, 11647.