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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, 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 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 leads 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 (see http://www.nibb.ac.jp/evodevo/0.TopE.html) and is feasible for use in experiments. Cells in a dissected leaf are reprogrammed to become chloronema apical stem cells within 24 hours. We reported that two paralogous *P. patens WUSCHEL-related homeobox 13-like* (*PpWOX13L*) genes, homologs of stem cell regulators in flowering plants, are transiently upregulated and required for the initiation of cell growth during stem cell formation. Concordantly, Δ*ppwox13l* deletion mutants fail to upregulate genes encoding homologs of cell wall loosening factors during this process. During the moss life cycle, most of the Δ*ppwox13l* mutant zygotes fail to expand and initiate an apical stem cell to form the embryo. Our data show that *PpWOX13L* genes are required for the initiation of cell growth specifically during stem cell formation, in analogy to WOX stem cell functions in seed plants, but using a different cellular mechanism (Sakakibara *et al*. 2014).

To perform fine live imaging during the reprogramming process, we now try to apply Adaptive optics (AO) to the observation of Physcomitrella cells. AO is the technique to cancel the aberration caused by atmospheric turbulence and to perform diffraction- limited observation of celestial bodies from the ground. Applying AO to microscopy, we can cancel the aberration caused by cellular structures and perform high-resolution live imaging. To construct the AO system, we first analyzed the optical properties of Physcomitrella cells. Live-cell imaging with bright field and phase contrast microscopies as well as image degradation analysis using fluorescent beads demonstrated that chloroplasts are the main source of the disturbance in the cell (Tamada *et al*. 2014). According to this information, we constructed a prototype of an AO microscope that can correct the aberration caused by chloroplasts. Images of chloroplasts that are located at the opposite side from the objective lens in the leaf cell are degraded due to aberration from cellular structures including chloroplasts themselves. With AO microscopy, we successfully obtained fine images of the chloroplast where the grana structure was observed more clearly. This study was mainly conducted by Yosuke Tamada.

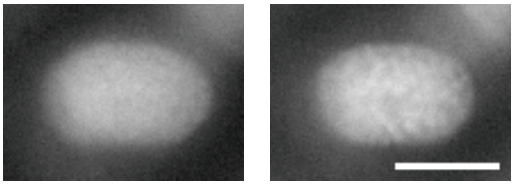

Figure 1. AO correction of a chloroplast image. Left, no correction; right, with AO. Bar, 5 μm.

III. Evolution of Regeneration: Master Regulator for Reprogramming STEMIN

Animal somatic cells can be reprogrammed to induce pluripotent stem (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. On the other hand, differently from *STEMIN1*, induction of *STEMIN2* or *STEMIN3* in gametophores did not change leaf cells into chloronema apical stem cells. These indicate that *STEMIN1* has an enough ability to change intact leaf cells to stem cells, but its paralogous genes do not. Masaki Ishikawa was this study's main researcher.

IV. Evolution of Elaborated Cell Division Machinery: Spindle body

At mitosis, all eukaryotic cells divide chromosomes to two daughter cells using a mitotic spindle, which is composed of microtubules. For accurate distribution of the chromosomes, the spindle has two poles. The centrosomes, which act as microtubule organizing centers, ensure formation of the two poles in metazoan cells. In contrast, the cells of land plants and their sister group, charophycean green algae, form a 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. It is challenging, however, to visualize microtubule reorganization during spindle formation in living plant cells, because large numbers of microtubules rapidly redistribute in 3-dimensional space. 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. Our data shows that microtubules elongate from template microtubules on the nuclear envelope upon nuclear envelope breakdown. The template microtubules are distinct from the spindle microtubules because they disappear during spindle development. The data suggests that microtubules which had been organized before spindle formation are the organizers of the bipolar spindle. Takashi Murata was this study's main researcher.

V. Evolution of water conducting systems

The development of a water conducting system was one of the most important requirements for land plants. Recent studies implicate a group of NAC domain transcription factors including VND6 and VND7 of *Arabidopsis thaliana* as key regulators of formation of xylem vessels, water-conducting cells in vascular plants. However, molecular mechanisms for development of other types of water-conducting cells are still unclear. We collaborated with Prof. Taku Demura in NAIST and showed that their *Physcomitrella patens* homologues, named PpVNS1 to PpVNS8, play a crucial role during the development of hydroids, specialized water-conducting cells in bryophytes. *PpVNS* genes are expressed in the midrib of developing leaves, in which hydroids are developed. Overexpression of *PpVNS* genes induced cell death in *P. patens* and ectopic formation of vessel-like cells in the vascular plant *Arabidopsis*. From these observations, we proposed that the last common ancestor of bryophytes and vascular plants had developed a system using VNS homologues to induce cell death during water-conducting cell formation. Our findings also suggest that the water-conducting cells in bryophytes and vascular plants are homologous, and that current land plants share a conserved genetic basis with VNS family proteins for development of water conducting systems (Xu *et al*. 2014).

VI. 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 histological, physiological, biochemical, and behavioral approaches, the lack of reverse 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). This new technique is currently applied for live imaging of actin cytoskeleton and calcium dynamics, both of which participate in the seismonastic movement through as-yet-unidentified mechanisms. Our transgenic technique also enabled us to develop a CRISPR/Cas-mediated gene knock-out system in this species. To investigate adaptive meanings of the seismonastic movement, we now try to produce immotile mutants using the CRISPR/Cas system. This study was conducted mainly by Hiroaki Mano.

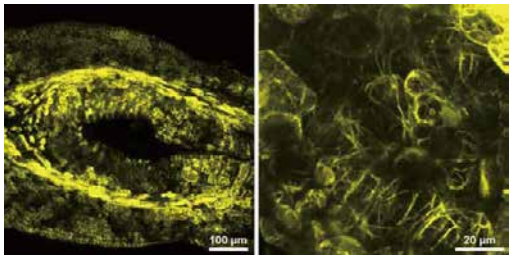

Figure 2. Visualization of actin cytoskeleton in leaflets of *Mimosa pudica* using a Lifeact-Venus protein

VII. 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. On the other hand, the oxidized form of xanthommatin was found in mantises with brown body color. To further elucidate the mechanism underlying

the different body coloration, we are now analyzing the ultrastructure of ommochrome granules in pigment cells of these mantises by transmission electron microscopy. This work was mainly done by Hiroaki Mano.

VIII. Evolution of pitcher leaves in carnivorous plants

Carnivorous plants form specialized leaves that are capable of attracting, trapping, and digesting prey and absorbing nutrients. The unusual plants evolved from non-carnivorous plants but their evolutionary process is mostly unknown. To understand the genomic changes associated with the evolution of carnivory, we sequenced 2-Gbp genome of the Australian pitcher plant *Cephalotus follicularis* in collaboration with Beijing Genomics Institute. This study was conducted mainly by Kenji Fukushima and Tomoko Shibata.

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