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All living organisms evolved from a common ancestor that lived more than 3.5 billion years ago, and the accumulation of mutations in their genomes has resulted in the present biodiversity. Traces of the evolutionary process are found in the genomes of extant organisms. By comparing the gene networks (and their functions) of different organisms, we hope to infer the genetic changes that caused the evolution of cellular and developmental processes.

I. Evolution from unicellular to multicellular organisms

The first evolutionary step from unicellular to multicellular organisms is the formation of two different cells from a single cell via asymmetric cell division. The first cell division of a protoplast isolated from the protonemata of the moss *Physcomitrella patens* is asymmetric regarding its shape and nature, and gives rise to an apical pluripotent stem cell and a differentiated protonema cell. A systematic overexpression screening for genes involved in asymmetric cell division of protoplasts in *P. patens* was performed for 4,000 full-length cDNA clones. We identified 58 cDNAs whose overexpression caused defects in asymmetric cell divisions and their functional analyses are in progress. This work was performed as a collaboration with Dr. Tomomichi Fujita (Hokkaido University).

II. Evolution from cells to tissues based on molecular mechanisms of cytokinesis

The cells of land plants and their sister group, charophycean green algae, divide by the insertion of cell plates at cytokinesis. This is in contrast to other green algae, in which

the invagination of the plasma membrane separates daughter cells at cytokinesis. The cell plate appears in the middle of daughter nuclei, expands centrifugally towards the cell periphery, and finally fuses to the parental cell wall. Cell wall materials are transported to the expanding cell plate with a phragmoplast, which is mainly composed of microtubules. Centrifugal expansion of the phragmoplast is a driving force for that of the cell plate, although elucidating the molecular mechanism for the centrifugal expansion of the phragmoplast was a challenge. Based on live imaging of α -tubulin at a light microscopic level and immunolocalization of γ -tubulin at an electron microscopic level, we proposed a hypothesis that cytosolic γ -tubulin complexes are recruited onto existing phragmoplast microtubules and nucleate new microtubules as branches, and that the branched microtubules drive phragmoplast expansion. Seeing the life history of microtubules in the phragmoplast had been very difficult by live imaging of α -tubulin, but we successfully tracked the trajectories of growing microtubule ends in the phragmoplast using two-photon microscopy of a microtubule plus-end marker EB1. Microtubules appeared in many sites in the phragmoplast and elongated obliquely towards the cell plate. We also found that inhibition of γ -tubulin function by antibody injection inhibited formation of new microtubules and phragmoplast expansion. These results support our hypothesis. Takashi Murata was this study's main researcher.

Microtubules form arrays with parallel and antiparallel bundles and function in various cellular processes including cell division. The interdigitation of antiparallel microtubules in phragmoplasts, plant-unique microtubules arrays, is formed and stabilized by microtubules-associated proteins (MAPs) including kinesins. However, mechanisms for disassembly of the interdigitation of antiparallel microtubules are still unclear. Here we show a type II ubiquitin-like domain protein, PUBL1 and PUBL2 (for *Physcomitrella patens* *ubiquitin-like domain protein 1 and 2*), are novel factors regulating disassembly of the antiparallel bundles and depolymerization of the microtubules in the phragmoplasts. PUBL1 and PUBL2 proteins were predominantly localized to the interdigitation of the antiparallel microtubules. In the double-deletion lines of both genes, the collapse of the interdigitating microtubules in the phragmoplasts was retarded, indicating that PUBL1 and PUBL2 are indispensable for proper loss of the interdigitation. A kinesin, KIN1D1a, for generation of the interdigitation aberrantly persisted in the phragmoplast equator, suggesting that the crosslink between the plus ends of the antiparallel microtubules is properly lost in the double-deletion lines. Furthermore, double-deletion lines exhibited formation of incomplete cell plate and multinucleated cells, suggesting that PUBL1 and PUBL2, the proper disassembly of the interdigitation, or both are required for proper lateral expansion of the cell plate. Yuji Hiwatashi mainly performed this study.

III. Evolution of molecular mechanisms in plant development

3-1 Stem cell initiation and maintenance

The initiation and maintenance of several types of stem cells to produce different types of differentiated cells is precisely regulated during the development of multicellular organisms. Molecular mechanisms for stem cell characterization, however, have remained largely unknown. We showed that *AINTEGUMENTA/PLETHORA/BABY BOOM (APB)* orthologs *PpAPBs* (*PpAPB1*, 2, 3, and 4) are involved in stem cell characterization in the moss *Physcomitrella patens*. Gametophore stem cells were induced by exogenous cytokinin in the wild type, while the quadruple disruptants did not form any gametophore stem cells with exogenous cytokinin application. These results suggest that the *PpAPBs* play a critical role in the characterization of a gametophore stem cell. Meanwhile, the expression of *PpAPBs* is regulated by auxin, not cytokinin. The primary researchers for this study were Tsuyoshi Aoyama and Yuji Hiwatashi.

3-2 Nuclear genome project of the moss *Physcomitrella patens*

A comparison of developmental genes among major land plant taxa would facilitate our understanding of their evolution, although it has not been possible because of the lack of genome sequences in basal land plants. We established an international consortium for a genome project of the moss *Physcomitrella patens* and the lycopod *Selaginella moellendorffii*. After publication of the draft genome of *Physcomitrella patens*, to further elaborate the contig assembling and the gene annotation, we performed (1) EST analyses of several libraries of cDNAs isolated from different developmental stages; (2) construction of full-length cDNA libraries and sequencing in their full length; (3) construction of BAC libraries and their end-sequencing; (4) 5'-end serial analysis of gene expression (5' SAGE); and (5) a collection of 3' UTR and small RNA sequences as collaborative works with groups associated with Dr. Tomoaki Nishiyama (Kanazawa Univ.), Prof. Asao Fujiyama (National Institute of Informatics), Prof. Sumio Sugano (Univ. Tokyo), and Prof. Yuji Kohara (National Institute of Genetics).

We developed a system to efficiently construct phylogenetic trees with whole genome shotgun sequence data in public databases before their assembly. We collected homologs of approximately 700 *Arabidopsis thaliana* genes involved in development, and their phylogenetic analyses are in progress.

3-3 Functional characterization of polycomb genes in the moss *Physcomitrella patens*

Land plants have distinct developmental programs in haploid (gametophyte) and diploid (sporophyte) generations. Although usually the two programs strictly alternate at fertilization and meiosis, one program can be induced during the other program. In a process called apogamy, cells of the gametophyte other than the egg cell initiate sporophyte development. Here, we report for the moss *Physcomitrella*

patens that apogamy resulted from deletion of the gene orthologous to the *Arabidopsis thaliana* *CURLY LEAF (PpCLF)*, which encodes a component of polycomb repressive complex 2 (PRC2). In the deletion lines, a gametophytic vegetative cell frequently gave rise to a sporophyte-like body. This body grew indeterminately from an apical cell with the character of a sporophytic pluripotent stem cell but did not form a sporangium. Furthermore, with continued culture, the sporophyte-like body branched. Sporophyte branching is almost unknown among extant bryophytes. When PpCLF was expressed in the deletion lines once the sporophyte-like bodies had formed, pluripotent stem cell activity was arrested and a sporangium-like organ formed. Supported by the observed pattern of PpCLF expression, these results demonstrate that, in the gametophyte, PpCLF represses initiation of a sporophytic pluripotent stem cell and, in the sporophyte, represses that stem cell activity and induces reproductive organ development. In land plants, branching, along with indeterminate apical growth and delayed initiation of spore-bearing reproductive organs were conspicuous innovations for the evolution of a dominant sporophyte plant body. Our study provides insights into the role of PRC2 gene regulation for sustaining evolutionary innovation in land plants. Yosuke Okano and Takaaki Ishikawa were this study's primary researchers.

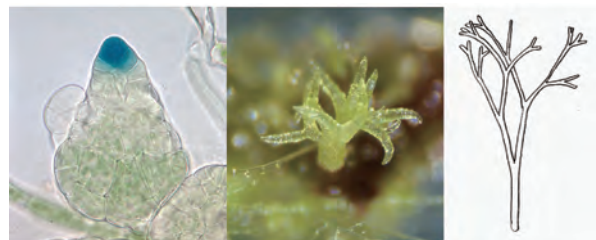


Figure 1. A *PpCURLY LEAF* deletion mutant formed sporophyte-like bodies with expression of a sporophyte stem cell specific MKN4 gene (left). A sporophyte-like body formed branches (center), which is similar to extinct prototracheophytes (right).

IV. Molecular mechanisms of female and male interactions

In sexual reproduction, proper communication and cooperation between male and female organs and tissue are essential for male and female gametes to unite. In flowering plants, female sporophytic tissues and gametophyte direct a male pollen tube towards an egg apparatus, which consists of an egg cell and two synergid cells. The cell-cell communication between the pollen tube and the egg apparatus, such as the reception of a signal from the egg apparatus at the pollen tube, makes the tip of pollen tube rupture to release the sperm cell. To detect male factors involved in this communication, we screened mutants of receptor-like kinases expressed in pollen tubes and characterized the *ANXURI (ANX1)* and *ANXUR2 (ANX2)* genes. Here we report that pollen tubes of *anx1/anx2* ruptured before arriving at the egg apparatus, suggesting that *ANX1* and *ANX2* are male factors controlling pollen tube

behavior by directing rupture at the proper timing. Furthermore, *ANX1* and *ANX2* were the most closely related paralogs to a female factor, *FERONIA/SIRENE*, controlling pollen tube behavior expressed in synergid cells. Our finding shows that the coordinated behaviors of female and male reproductive apparatuses are regulated by the sister genes, whose duplication might play a role in the evolution of fertilization system in flowering plants. This work was mainly done by Saori Miyazaki.

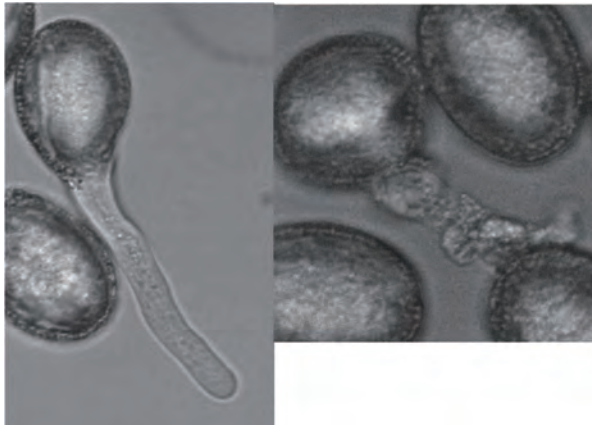


Figure 2. Pollen tubes of wild type (left) and the *anxl;anx2* mutant (right) on culture medium. Ruptured *anxl;anx2* pollen tube released sperm nuclei together with pollen tube cytoplasm before fertilization.

V. Molecular mechanisms of mimicry

Mimicry is an intriguing phenomenon in which an organism closely resembles another, phylogenetically distant species. An excellent example is the flower-mimicry of the orchid mantis *Hymenopus coronatus*, in which pink and white coloration and petal-like structures on its walking legs enable this insect to blend perfectly into flowers. To elucidate the evolutionary mechanism of this complex mimicry at the molecular level, we first focused on the mechanism of body coloration in the orchid mantis. HPLC and mass spectrometric analyses suggested that xanthommatin, a red pigment belonging to the ommochrome family, contributes to the pink body coloration of the mantis. We also found that the orchid mantis contains large amounts of leucopterin and isoxanthopterin, both of which are known as white compounds in other insects such as *Pierid* butterflies. These results indicate that the “flower-like” coloration of the orchid mantis is formed by pigments unrelated to those used for the coloration of flowers.

The orchid mantis drastically changes its appearance during post-hatching development. The first-instar nymph of the mantis is colored red and black and is believed to mimic other unpalatable insects like ants. A flower-like appearance emerges after molting into the 2nd-instar nymph. We aim to compare the gene expression profiles between the 1st- and 2nd-instar nymphs using a high-throughput DNA sequencer. This work was mainly done by Hiroaki Mano.

VI. Molecular mechanisms of host shifting

Although plant-feeding insects as a whole utilize various plant species, the majority of plant-feeding insect species are associated with one or a few plant species. Such mono- and oligophagous insect species are highly specialized to their respective host plant species via larval physiological adaptation (assimilability) and host preference of adult females. Thus, the process of host shifting to a novel plant species involves the evolution of multiple traits. The molecular mechanisms underlying such multi-trait evolution are largely unknown. To address the molecular mechanism of host shifting, we use two host races in a tiny moth, *Acrocercops transecta*, as a model system. Host races in plant-feeding insects are subpopulations that are specialized to different species of host plants, so we can conduct QTL analyses of the host-adaptation traits by crossing the two host races. The segregation patterns of larval assimilability and ovipositing female preference in F2 and backcross generations indicated that the two traits were governed by a few major loci, but were under different genetic control. To test whether these loci are physically linked with each other or not, mapping analyses are in progress. This study was conducted mainly by Issei Ohshima.

Publication List

[Original papers]

- Kofuji, R., Yoshimura, T., Inoue, H., Sakakibara, K., Hiwatashi, Y., Kurata, T., Aoyama, T., Ueda, K., and Hasebe, M. (2009). Gametangia development in the moss *Physcomitrella patens*. In *The Moss Physcomitrella patens*, D. Cove, F. Perroud, and C. Knight, eds. (Wiley-Blackwell), pp. 167-181.
- Miyazaki, S., Murata, T., Sakurai-Ozato, N., Kubo, M., Demura, T., Fukuda, H., and Hasebe, M. (2009). *ANXUR1* and 2, sister genes to *FERONIA/SIRENE*, are male factors for coordinated fertilization. *Curr. Biol.* *19*, 1327-1331.
- Oda, Y., Hirata, A., Sano, T., Fujita, T., Hiwatashi, Y., Sato, Y., Kadota, A., Hasebe, M., and Hasezawa, S. (2009). Microtubules regulate dynamic organization of vacuoles in *Physcomitrella patens*. *Plant Cell Physiol.* *50*, 855-868.
- Okano, Y., Aono, N., Hiwatashi, Y., Murata, T., Nishiyama, T., Ishikawa, T., Kubo, M., and Hasebe, M. (2009). A polycomb repressive complex 2 gene regulates apogamy and gives evolutionary insights into early land plant evolution. *Proc. Natl. Acad. Sci. USA* *106*, 16321-16326.