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I. The lycopod *Selaginella moellendorffii* genome

Vascular plants appeared ~410 million years ago, then diverged into several lineages of which only two survive: the euphyllophytes (ferns and seed plants) and the lycophytes. We report here the genome sequence of the lycophyte Selaginella moellendorffii (Selaginella), the first nonseed vascular plant genome reported. By comparing gene content in evolutionarily diverse taxa, we found that the transition from a gametophyte- to a sporophyte-dominated life cycle required far fewer new genes than the transition from a nonseed vascular to a flowering plant, whereas secondary metabolic genes expanded extensively and in parallel in the lycophyte and angiosperm lineages. Selaginella differs in posttranscriptional gene regulation, including small RNA regulation of repetitive elements, an absence of the trans-acting small interfering RNA pathway, and extensive RNA editing of organellar genes.

Figure 1. A lycopod *Selaginella moellendorffii* whose draft genome was published.

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 expansion was a challenge. We have found that γ-tubulin complexes on existing phragmoplast microtubules nucleate new microtubules as branches. Although elongation of the branched microtubules is likely a driving force of the phragmoplast expansion, the mechanism by which phragmoplast microtubules redistribute towards the cell periphery is unclear. Because an inhibitor of microtubule depolymerization inhibits phragmoplast expansion, analyses of microtubule depolymerization might be a key for understanding the mechanism. We developed a method for quantifying the rate of microtubule depolymerization in the phragmoplast, and found that the rate of microtubule depolymerization gradually increases from the outer surface to the inside of the phragmoplast. Based on the results, we propose a hypothesis that random branching of microtubules coupled with biased depolymerization lead directional redistribution of microtubules, which drives centrifugal expansion of the phragmoplast. Takashi Murata was this study's main researcher.

III. Evolution of molecular mechanisms in plant development

Stem cells are formed at particular times and positions during the development of multicellular organisms. Whereas flowering plants form stem cells only in the sporophyte generation, non-seed plants form stem cells in both the sporophyte and gametophyte generations. Although the molecular mechanisms underlying stem cell formation in the sporophyte generation have been extensively studied, only a few transcription factors involved in the regulation of gametophyte stem cell formation have been reported. The

moss *Physcomitrella patens* forms a hypha-like body (protonema) and a shoot-like body (gametophore) from a protonema apical cell and a gametophore apical cell, respectively. These apical cells have stem cell characteristics. We found that four AP2-type transcription factors orthologous to *Arabidopsis thaliana AINTEGUMENTA/ PLETHORA/ BABY BOOM* (*APB*) are indispensable for the formation of gametophore apical cells from protonema cells. Quadruple disruption of all *APB* genes blocked gametophore formation, even in the presence of cytokinin, which enhances gametophore apical cell formation in the wild type. Heat-shock induction of an *APB4* transgene driven by a heat-shock promoter increased the number of gametophores. Expression of all APB genes was induced by auxin but not by cytokinin. Thus, the APB genes function synergistically with cytokinin signaling to determine the identity of the two types of stem cells. The primary researchers for this study were Tsuyoshi Aoyama and Yuji Hiwatashi.

Flowers are the most complex reproductive organs in land plants, whose development is regulated by MADS-box transcription factors. To understand the origin of a genetic network of floral homeotic genes, we analyzed six MIKC classic type MADS-box genes in *P. patens*. Deletion of all six genes enhanced elongation of gametophore stem and reduced production of sporophytes. This result suggests that the MADS-box genes function in both gametophyte and sporophyte generation. Investigation of the effects of all six gene deletions is currently being undertaken by Yuji Hiwatashi.

Evolution of a branched system is a conspicuous novelty in land plant evolution, although the origin and evolution of its gene network is not known because of the lack of study in the basal land plants. We found that a deletion mutant of a polycomb repressive complex 2 gene *PpCLF* forms a branched sporophyte-like organ in *P. patens*. Analyses of auxin distribution and expression patterns of class 1 KNOX genes suggest that the active site of auxin signaling is localized to the initiation site of the branch. To elucidate how the active site is formed in the sporophyte-like organ, spatial expression patterns of the proteins related to auxin biosynthesis, inactivation, and transport are under investigation. This work was mainly done by Yuji Hiwatashi.

IV. Molecular mechanisms of reprogramming of gametophore leaf cells to pluripotent stem cells in the moss *Physcomitrella patens*

Differentiated cells can be reprogrammed to become undifferentiated pluripotent stem cells with abilities to both self-renew and give rise to most cell types in the organism. An induction of reprogramming is more easily manipulated in plants than in animals, although the genetic and molecular bases of the difference are mostly unknown. This is likely because the callus usually used in reprogramming studies in seed plants is a cell mixture composed of reprogrammed and unreprogrammed cells. We noticed that *P. patens* should overcome this problem by its rapid reprogramming ability from a single cell (see http://www.nibb.ac.jp/evodevo/ERATO/movie/MacMovie.mp4). Cells in a dissected leaf of *P. patens* are reprogrammed to become chloronema apical cells with pluripotency within 24 hours. We can continuously observe the reprogramming process of a specific cell under a microscope.

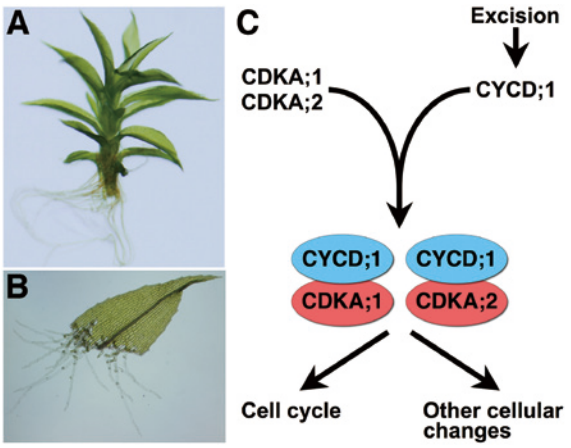

Figure 2. Reprogramming of leaf cells to chloronema apical cells. (A) A gametophore. (B) A leaf at 48 hours after excision. (C) A model showing the dual roles of cyclin-dependent kinase A (CDKA) during reprogramming.

One of the key factors of reprogramming is the change in the genome-wide chromatin modification. In the differentiated cells, a gene expression profile that fits the cell function is stably maintained through chromatin modifications. The active modifications such as trimethylation of histone H3 at lysine 4 (H3K4me3) are enriched at certain genes required for the cell function, and the repressive modifications including H3K27me3 are enriched at other unnecessary genes. In contrast, in the pluripotent stem cells, most genes are ready to be activated. Many genes with H3K27me3 also have H3K4me3 in animal pluripotent stem cells, and this bivalent state is presumed to keep genes poised for transcription. Thus, in the process of reprogramming, the epigenomic profile of differentiated cells should be changed into a pluripotent stem cell-specific epigenomic profile. However, the mechanisms of the establishment of such epigenomic profiles are almost unknown. We are currently attempting to reveal these mechanisms underlying reprogramming of *P. patens* leaf cells to chloronema apical cells with the combination of chromatin immunoprecipitation-sequencing (ChIP-Seq) using a next generation sequencer and live imaging of chromatin modifications. We have successfully established the 4D (3D + time) live-imaging method of a single *P. patens* nucleus, and also produced the H3K27me3 detector using *Drosophila melanogaster* Polycomb protein, which is known to bind to H3K27me3. We are now analyzing the ChIP-Seq data, performing the 4D live imaging for H3K27me3 during the reprogramming, and producing the live-imaging detector for H3K4me3. This study is mainly conducted by Takaaki Ishikawa and Yosuke Tamada.

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 indicated that xanthommatin, a common red pigment of the ommochrome family, almost solely contributes to the pink body coloration of late-stage nymphs. On the other hand, the 1st-instar nymph of the orchid mantis with yellowish red color contains three ommochrome pigments; xanthommatin, decarboxylated xanthommatin and a labile yellow pigment which was previously uncharacterized. These results suggest that the orchid mantis alters its body color by changing the composition of ommochrome pigments during post-hatching development. This work was mainly done by Hiroaki Mano.

VI. Molecular mechanisms of host shifting

Adaptation to a novel environment often requires evolution of multiple traits. In phytophagous insects a precise combination of performance and preference traits for particular host plants is crucial for host shifting because a new host plant can be incorporated into an insect's diet if adults accept it for oviposition and if the larvae are able to complete their development on it. However, very little is known about the genetic bases of the performance and preference, which are fundamental to infer the process and evolutionary consequence of host shifting. To address the molecular mechanism of host shifting, we use two host races of a tiny moth, *Acrocercops transecta*, as a model system. A QTL analysis revealed that only a restricted region of a single autosome was responsible for the larval performance. This indicates that host shifting from *Juglans* to *Lyonia* in *A. transecta* involved changes in few genes with large effect, suggesting that a small number of genetic changes to larval performance allowed the successful host shifting. To test whether preference genes are physically linked with performance genes or not, a mapping analysis of preference genes is in progress. This study was conducted mainly by Issei Ohshima.

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

The molecular mechanisms and evolutionary significance of plant movement, including seismonastic and nyctinastic movements, are enigmatic. We are working to establish chemically mutagenized lines that lack movement to compare fitness to wild types. We are also attempting to set up a method for transformation to characterize the genes involved in movement. To achieve this goal, we use a cotyledonary node explant, which can regenerate multiple shoots in the presence of 6-benzylaminopurine (BAP), as a target of Agrobacterium-mediated gene transfer. Although the node explant is highly recalcitrant to Agrobacterium infection, we successfully obtained several lines of transformed calluses that were capable of developing new shoots. We are now trying to regenerate whole plants from these transformed shoots in addition to further improvement of transformation efficiency. This study was conducted mainly by Hiroaki Mano.

VIII. Evolution of pitcher leaves in carnivorous plants

Development and evolution of the unique morphology of pitcher-shaped leaves of the carnivorous plant family Sarraceniaceae remains problematic. Since the 1870's, the pitcher leaves have been hypothesized to have a similar developmental program to that of peltate leaves. However, this hypothesis could not explain the formation of the keel, a structure specific to pitcher leaves. To understand the development and evolution of pitcher leaves, we analyzed expression patterns of leaf developmental gene orthologs in *Sarracenia purpurea*. Unexpectedly, the results suggested that adaxial-abaxial patterning of pitcher leaves was different from those of peltate leaves and have enabled us to hypothesize the evolutionary process of pitcher leaves. This study was conducted mainly by Kenji Fukushima.

Publication List

〔Original papers〕

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- Banks, J.A., Nishiyama, T., Hasebe, M., Bowman, J.L., Gribskov, M., dePamphilis, C., Albert, V.A., Aono, N., Aoyama, T., Ambrose, B.A., *et al*. (2011). The Selaginella genome identifies genetic changes associated with the evolution of vascular plants. Science *332*, 960-963.
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- Motose, H., Hamada, T., Yoshimoto, K., Murata, T., Hasebe, M., Watanabe, Y., Hashimoto, T., Sakai, T., and Takahashi, T. (2011). NIMA-related kinases 6, 4, and 5 interact with each other to regulate microtubule organization during epidermal cell expansion in *Arabidopsis thaliana*. Plant J. *67*, 993-1005.