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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 meristematic cell and a differentiated non-meristematic 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 plasma membrane separates daughter cells at cytokinesis. The cell plate appears in the middle of daughter nuclei, expands centrifugally towards a cell periphery, and finally fuses to a parental cell wall. Cell wall materials are transported to the expanding cell plate with a phragmoplast, which is mainly composed of microtubules. A 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.

In addition to the centrifugal expansion, antiparallel bundles in phragmoplasts are mostly unexplored and potentially offer new cellular insights. We found that the *Physcomitrella patens* kinesins KINID1a and KINID1b (for kinesin for interdigitated microtubules 1a and 1b), which are specific to land plants and orthologous to *Arabidopsis thaliana* PAKRP2, are novel factors indispensable for the generation of interdigitated antiparallel microtubules in the phragmoplasts of the moss *P. patens*. KINID1a and KINID1b are predominantly localized to the putative interdigitated parts of antiparallel microtubules. This interdigitation disappeared in double-deletion mutants of both genes, indicating that both KINID1a and 1b are indispensable for interdigitation of the antiparallel microtubule array. Furthermore, cell plates formed by these phragmoplasts did not reach the plasma membrane in approximately 20% of the mutant cells examined. We observed that in the double-deletion mutant lines,

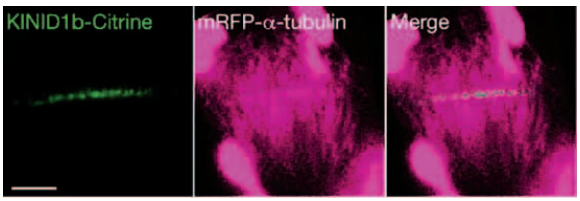

Figure 1. Localization of KINID1b-Citrine and mRFP-α-tubulin fusion proteins. The fluorescence derived from the mRFP-α-tubulin was of higher intensity at the phragmoplast equator where the plus ends of the antiparallel microtubules interdigitate. Fluorescent signals of KINID1b-Citrine protein overlapped with the high intensity mRFP signals. A bar = 2 μm.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

chloroplasts remained between the plasma membrane and the expanding margins of the cell plate, while chloroplasts were absent from the margins of the cell plates in the wild type. This suggests that the kinesins, the antiparallel microtubule bundles with interdigitation, or both are necessary for the proper progression of cell wall expansion.

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 properly regulated during the development of multicellular organisms. Molecular mechanisms for the stem cell characterization, however, have been 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.

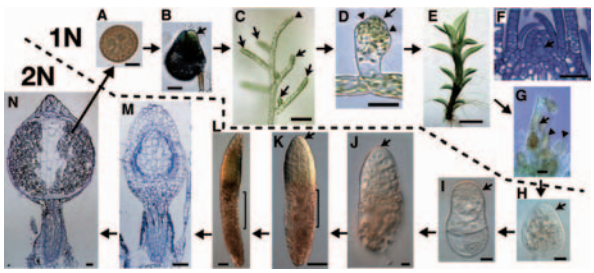

Figure 2. Life cycle of the moss *Physcomitrella patens*. Arrows indicate stem cells in different organs (see Sakakibara et al. 2008 in detail.)

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 was not possible because of the lack of genome sequences in basal land plants. We established an international consortium for a genome project of the basal land plant; subsequently, the moss *Physcomitrella patens* and its entire genome has been mostly sequenced as a collaborative work with the international consortium (Rensing et al. 2008).

To further elaborate the contig assembling and the gene annotation, we performed (1) the EST analyses of several libraries of cDNAs isolated from different developmental stages; (2) the construction of full-length cDNA libraries and sequencing in their full length; (3) the 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 available 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*

Polycomb group (PcG) proteins regulate chromatin modification and function as a cellular memory system to maintain the repressed state of developmental genes in both animals and plants. PcG genes are involved in phase changes of *Arabidopsis thaliana* development, such as vegetative to reproductive and haploid to diploid transitions. Bryophytes have dominant haploid generation, while sporophyte generation is dominant in angiosperms. The change of dominant generations is one of the most conspicuous evolutionary aspects of land plants. To elucidate the molecular mechanisms underlying the evolution in alteration of generations, the characterization of PcG genes in *P. patens* is in progress. Takaaki Ishikawa and Yosuke Okano were this study's primary researchers.

Ⅳ. Molecular mechanisms of female and male interactions

Sexual isolation is an important step for speciation, although the molecular mechanisms governing the isolation in plants are mostly unknown. A proper pollen tube guidance is essential for reproduction in angiosperms, and sexual isolation is often related to the arrest of guidance. In spite of the long history of studies on the pollen tube guidance, few guidance factors have been reported because of the difficulty of genetic analyses. We focused on receptors like kinases (RLKs), which function to receive extra cellular ligands and transmit the signal into a cell. We postulated that RLKs involved in pollen tube guidance are likely expressed more abundantly in pollen and/or pollen tube than in other tissue. Gene expression profiles between A. *thaliana* pollen and pollen tube were compared with those of other tissues using microarray. Pollen and pollen tube expression profiles were similar to each other and 95 % of expressed genes were overlapped within the 4 fold differences. We focused on 45 RLKs predominantly expressed in pollen or germinating pollen to characterize signaling mechanisms during fertilization. Characterization of single and double T-DNA insertion lines are in progress. This work was mainly done by Saori Miyazaki.

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, in which pink and white coloration and petal-like structures on its walking legs enable this insect to blend perfectly into flowers. We attempted to elucidate the evolutionary mechanism of this complex mimicry at the molecular level. We first focused on the orchid mantis's mechanism of body coloration. Mass spectrometric analysis suggested that dihydro-xanthommatin, a red pigment belonging to the ommochrome family, contributes to the pink body coloration of the mantis. We also isolated a cDNA encoding a key enzyme for biosynthetic pathway of ommochromes. Using the sequence of this gene, we are now trying to establish RNAi-mediated gene knock-down in the orchid mantis.

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 the 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 for 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〕

- Hiwatashi, Y., Obara, M., Sato, Y., Fujita, T., Murata, T., and Hasebe, M. (2008). Kinesins are indispensable for interdigitation of phragmoplast microtubules in the moss Physcomitrella patens. Plant Cell *20*, 3094-3106.
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- Inouye, T., Odahara, M., Fujita, T., Hasebe, M., and Sekine, Y. (2008). Expression and complementation analyses of a chloroplast-localized homolog of bacterial RecA in the moss Physcomitrella patens. Biosci. Biotechnol. Biochem. *72*, 1340-1347.
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- Abe, J., Hiwatashi, Y., Ito, M., Hasebe, M., and Sekimoto, H. (2008). Expression of exogenous genes under the control of endogenous HSP70 and CAB promoters in the Closterium peracerosum-strigosum-littorale complex. Plant Cell Physiol. *49*, 625-632.
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