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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 the 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 a generation of a new individual, which is an effective strategy for propagation. The ability of reprogramming 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/ERATO/movie/MacMovie.mp4) and is feasible for experiments. Cells in a dissected leaf are reprogrammed to become chloronema apical stem cells within 24 hours.

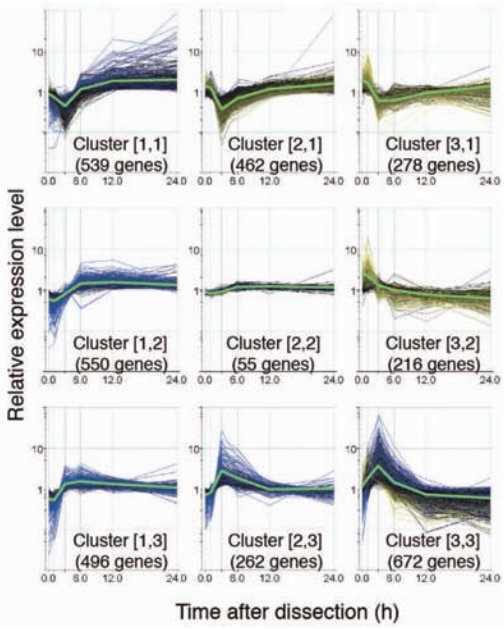

Figure 1. Expression changes of genes during reprogramming are classified into nine types. Reprogramming of leaf cells started at time 0.

To understand the underlying molecular mechanisms, a digital gene expression profiling method using mRNA 5'-end tags (5'-DGE) was established. The 5'-DGE method produced reproducible data with a dynamic range of four orders that correlated well with qRT-PCR measurements. After the excision of leaves, the expression levels of 10% of the transcripts changed significantly within 6 h. Genes involved in stress responses and proteolysis were induced and those involved in metabolism, including photosynthesis, were reduced. The later processes of reprogramming involved photosynthesis recovery and higher macromolecule biosynthesis, including of RNA and proteins. Comparison with stem cell formation via callus in flowering plants revealed that common phytohormone signaling pathways are activated during reprogramming, although no exogenous phytohormone is applied in the moss system, suggesting that an intrinsic phytohormone regulatory system may be used in the moss (Nishiyama et al. 2012).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

III. Evolution of Regeneration: Epigenetic regulation

In differentiated cells, 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, to ensure an expression profile that fits the cell function. In contrast, in animal pluripotent stem cells, most genes with H3K27me3 also have H3K4me3, and this bivalent chromatin state is presumed to keep genes poised for transcription and to be required for the pluripotency. Thus, changes in the genome-wide chromatin modifications must be associated with and required for the process of reprogramming. We are currently attempting to analyze the dynamics of the chromatin modifications in the reprogramming process of *P. patens* with the combination of chromatin immunoprecipitation-sequencing (ChIP-Seq) using a next generation sequencer and live imaging of chromatin modifications. We have successfully established a 4D (3D + time) live-imaging method of a single *P. patens* nucleus, and also produced a H3K27me3 detector using fluorescent protein fused to *Drosophila melanogaster* Polycomb protein, which is known to bind to H3K27me3. We are now analyzing the ChIP-Seq data, performing the 4D single-nucleus live imaging for H3K27me3 during the reprogramming, and producing live-imaging detector for other chromatin modifications including H3K4me3. This study was mainly conducted by Yosuke Tamada.

IV. 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. On the basis of the transcriptional profile during the moss reprogramming (Nishiyama et al., 2012), we selected genes, of which transcript levels are low in gametophores and up-regulated during reprogramming, of leaf cells after excision, and then overexpressed those genes in intact gametophores using the estrogen-inducible system. As a result, we identified a gene encoding a member of plant-specific transcription factor, STEM CELL-INDUCING FACTOR (STEMIN), that was able to induce direct reprogramming of differentiated leaf cells into chloronema apical stem cells without wounding signals. Using the 5'-DGE analysis, we found that many of the genes induced by the STEMIN-expression in intact gametophores overlapped with those upregulated during reprogramming in excised leaves. In addition, *STEMIN* promoter was activated at leaf cells that underwent reprogramming. Deletion of the *STEMIN* and its two paralogous genes delayed reprograming after leaf excision. Together, we suggest that STEMIN is a single master regulatory transcription factor governing *de novo* stem cell formation. Masaki Ishikawa was this study's main researcher.

V. Evolution of Elaborated Cell Division Machinery: Phragmoplast

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.

VI. Evolution of Developmental Programs

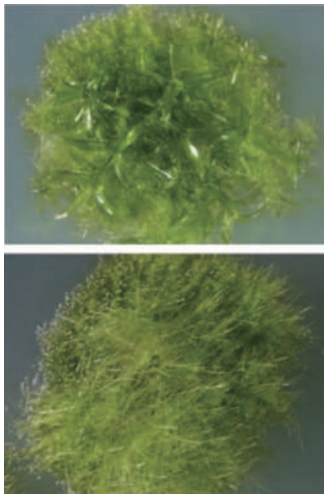

Figure 2. APB transcription factor is indispensable for a shoot body (gametophore) formation in the moss *Physcomiterlla patens*. Deletion of four APB genes caused no gametophores (lower panel) in comparison to wild type (upper panel).

The basal land plant mosses have two different developmental processes in the haploid generation, forming hypha-like protonemata and shoot-like gametophores. 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. 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 (Aoyama et al. 2012).

V. 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. HPLC and MS analyses indicated that xanthommatin, a common red pigment of the ommochrome family, almost solely contributes to the pink color of late-stage nymphs. On the other hand, 1st-instar nymph with yellowish red color contains a very labile pre-xanthommatin in addition to xanthommatin. 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

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. A QTL analysis of a tiny moth, *Acrocercops transecta* revealed that only a restricted region of a single autosome was responsible for the larval performance, suggesting that a small number of genetic changes to larval performance allowed the successful host shifting. Identification of the responsive genes is in progress with Dr. Issei Ohshima in Kyoto Prefecture University.

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. To introduce the sensitive plant *Mimosa pudica* as a model, we established a method for transformation. We used a cotyledonary node as a target of *Agrobacterium*-mediated gene transfer because of its ability of shoot regeneration. We obtained a large number of transformed calluses (55-60%) and succeeded in regenerating transgenic plants with a transformation efficiency of >5%. This study was conducted mainly 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. Whole-genome shotgun data corresponding to 100-fold depth were produced by Illumina paired-end/mate-pair sequencing with 180-bp to 5-kb insert sizes. A *de novo* assembly yielded a total of 1.7 Gbp in 43,308 scaffolds with 15.6 kb of contig N50 and 83.3 kb of scaffold N50. We further produced 14 Gbp of PacBio reads with 2-kb mean max subread length for gap filling. Transcript-based gene prediction with RNA-seq reads found 45,469 gene models. Genomic data enable us to deduce the origin and evolution of carnivory-related genes, such as digestive enzyme genes. This study was conducted mainly by Kenji Fukushima and Tomoko Shibata.

Publication List

〔Original papers〕

- Aoyama, T., Hiwatashi, Y., Shigyo, M., Kofuji, R., Kubo, M., Ito, M., and Hasebe, M. (2012). AP2-type transcription factors determine stem cell identity in the moss *Physcomitrella patens*. Development *139*, 3120-3129.
- Nishiyama, T., Miyawaki, K., Ohshima, M., Thompson, K., Nagashima, A., Hasebe, M., and Kurata, T. (2012). Digital gene expression profiling by 5'-end sequencing of cDNAs during reprogramming in the moss *Physcomitrella patens*. PLoS ONE *7*, e36471.
- Nishimura, T., Matano, N., Morishima, T., Kakinuma, C., Hayashi, K.I., Komano, T., Kubo, M., Hasebe, M., Kasahara, H., Kamiya, Y., and Koshiba, T. (2012). Identification of indole-3-acetic acid transport inhibitors including compounds affecting cellular PIN trafficking by two chemical screening approaches using maize coleoptile systems. Plant Cell Physiol. *53*, 1671-1682.
- Suetsugu, N., Sato, Y., Tsuboi, H., Kasahara, M., Imaizumi, T., Kagawa, T., Hiwatashi, Y., Hasebe, M., and Wada, M. (2012). The KAC family of kinesin-like proteins is essential for the association of chloroplasts with the plasma membrane in land plants. Plant Cell Physiol. *53*, 1854-1865.
- Zhao, N., Ferrer, J.L., Moon, H.S., Kapteyn, J., Zhuang, X., Hasebe, M., Stewart, C.N., Jr., Gang, D.R., and Chen, F. (2012). A SABATH Methyltransferase from the moss *Physcomitrella patens* catalyzes S-methylation of thiols and has a role in detoxification. Phytochemistry *81*, 31-41.