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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 use in experiments. Cells in a dissected leaf are reprogrammed to become chloronema apical stem cells within 24 hours. However, current systems for controlled transgene expression remain limited and we developed an estrogen receptor mediated inducible gene expression system, based on the system used in flowering plants (Kubo *et al*. 2013). After identifying the appropriate promoters to drive the chimeric transducer, we succeeded in inducing transcription over 1,000-fold after 24 h incubation with β-estradiol. The *P. patens* system was also effective for high-level long-term induction of gene expression; transcript levels of the activated gene were maintained for at least seven days on medium containing β-estradiol. We also established two potentially neutral targeting sites and a set of vectors for reproducible expression of two transgenes. This β-estradiol-dependent system will be useful to test genes individually or in combination, allowing stable, inducible transgenic expression in *P. patens*.

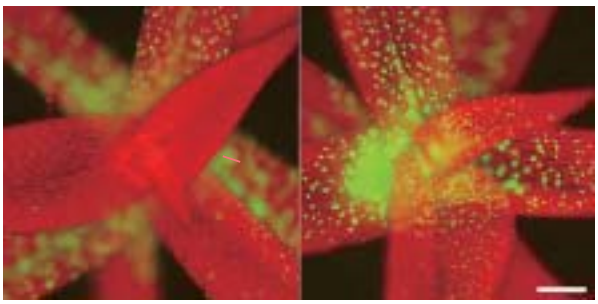

Figure 1. Spatial expression patterns of GFP induced by two different β-estradiol-dependent systems.

Genome-wide changes of chromatin modifications are required for the cell-fate transition including reprogramming to ensure a transcription profile that fits the new cell fate. However, the mechanisms and the timing of the changes during the cell-fate transition are still largely unclear. We analyzed genome-wide levels of active and repressive histone modifications, trimethylation of histone H3 at lysine 4 (H3K4me3) and H3K27me3, respectively, in the reprogramming process of *P. patens* with chromatin immunoprecipitation-sequencing (ChIP-seq). We revealed that the genome-wide changes occur at the last moment of the reprogramming process. We are currently attempting to specify the actual moment of the changes by performing 4D (3D + time) live imaging of chromatin modifications in a single *P. patens* nucleus. To detect H3K27me3, we introduced a gene encoding a fluorescent protein fused to a *Drosophila melanogaster* Polycomb protein, which binds to

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2013. The former title is indicated by an asterisk (*).

H3K27me3, into *P. patens*. We are now performing live imaging for H3K27me3 during the reprogramming, and producing novel detectors for other chromatin modifications including H3K4me3. This study was mainly conducted by Yosuke Tamada.

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. On the basis of the transcriptional profile during moss reprogramming (Nishiyama *et al*., 2012), we selected genes, of which transcript levels increase during reprogramming, and induced each candidate gene in gametophores using the estrogen inducible system. As a result, we identified a gene encoding a member of a 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. 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.

IV. 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. We found that the phragmoplast array comprises stable microtubule bundles and dynamic microtubules. We found that the dynamic microtubules are nucleated by γ-tubulin on stable bundles. The dynamic microtubules elongate at the plus ends and form new bundles preferentially at the leading edge of the phragmoplast. At the same time, they are moved away from the cell plate, maintaining a restricted distribution of minus ends. We propose that cycles of attachment of γ-tubulin complexes onto the microtubule bundles, microtubule nucleation and bundling, accompanied by minus-end-directed motility, drive the centrifugal development of the phragmoplast. Takashi Murata was this study's main researcher.

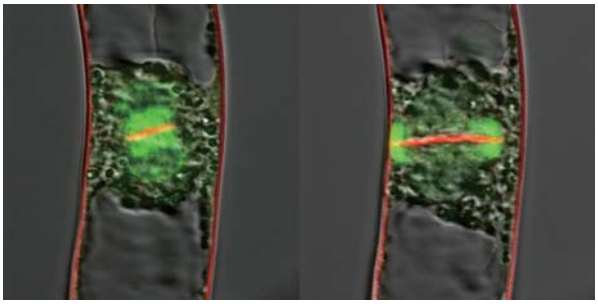

Figure 2. Centrifugal expansion of the phragmoplast. Green, phragmoplast; Red, cell plate and membrane.

V. Evolution of Life cycles

Land plants undergo an alternation of generations, in which multicellular bodies are produced in both haploid and diploid generations. Distinct developmental programs initiate after meiosis and fertilization in the haploid and diploid generations, respectively. Green alga, the sister to land plants, is inferred to have had a haplontic life cycle and the developmental program for the sporophyte generation was elaborated during land plant evolution. To understand the evolution of alteration of generations, we first analyzed molecular switches to start each generation. Previously we found that a polycomb repression complex 2 gene *CURLY LEAF* (*CLF*) represses initiation of sporophyte pluripotent stem cells in the gametophyte generation and the decrease of the protein was correlated to the start of the sporophyte generation (Okano *et al*. 2010 PNAS). We also found that *CLF* represses sporophyte stem cell activity in the sporophyte generation, and the timing of *CLF* expression regulates the length of the sporophyte generation and resultant body growth. This year, we found the other switch to start the haploid generation as a collaborative work with Drs. Keiko Sakakibara and John Bowman's groups in Hiroshima University and Monash University, respectively. Deletion of the class 2 KNOTTED1-LIKE HOMEOBOX (KNOX2) transcription factors in the moss *Physcomitrella patens* resulted in the development of gametophyte bodies from diploid embryos without meiosis. This indicates that KNOX2 acts to prevent the haploid-specific body plan from developing in the diploid plant body. Our findings indicate critical roles for the evolution of CLF and KNOX2 in establishing an alternation of generations in land plants.

VI. 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 and a mixture of ommochrome pigments were found in mantises with brown and yellowish-red color, respectively. These results suggest that the unique pink coloration of the orchid mantis is formed by the

predominance of the reduced form of xanthommatin. This work was mainly done by Hiroaki Mano.

VII. 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.

VIII. 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.

IX. 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 and PacBio sequencers. A *de novo* assembly yielded a total of 1.6 Gbp in 16,307 scaffolds with 99.5 kb of contig N50 and 287 kb of scaffold N50. Transcript-based and homology-based gene prediction with RNA-seq reads found 32,973 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〕

- Kim, S.Y., Colpitts, C.C., Wiedemann, G., Jepson, C., Rahimi, M., Rothwell, J.R., McInnes, A.D., Hasebe, M., Reski, R., Sterenberg, B.T., *et al*. (2013). Physcomitrella PpORS, basal to plant type III polyketide synthases in phylogenetic trees, is a very long chain 2'-oxoalkylresorcinol synthase. J. Biol. Chem. *288*, 2767-2777.
- Kubo, M., Imai, A., Nishiyama, T., Ishikawa, M., Sato, Y., Kurata, T., Hiwatashi, Y., Reski, R., and Hasebe, M. (2013). System for Stable beta-Estradiol-Inducible Gene Expression in the Moss. PLoS ONE *8*, e77356.
- Murata, T., Sano, T., Sasabe, M., Nonaka, S., Higashiyama, T., Hasezawa, S., Machida, Y., and Hasebe, M. (2013). Mechanism of microtubule array expansion in the cytokinetic phragmoplast. Nature Commun. *4*, 1967.
- Sakakibara, K., Ando, S., Yip, H.K., Tamada, Y., Hiwatashi, Y., Murata, T., Deguchi, H., Hasebe, M., and Bowman, J.L. (2013). KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants. Science *339*, 1067-1070.
- Shibata, T.F., Maeda, T., Nikoh, N., Yamaguchi, K., Oshima, K., Hattori, M., Nishiyama, T., Hasebe, M., Fukatsu, T., Kikuchi, Y., *et al*. (2013). Complete Genome Sequence of Burkholderia sp. Strain RPE64, Bacterial Symbiont of the Bean Bug Riptortus pedestris. Genome Announc. *1*, e00441-13.
- Zimmer, A.D., Lang, D., Buchta, K., Rombauts, S., Nishiyama, T., Hasebe, M., Van de Peer, Y., Rensing, S.A., and Reski, R. (2013). Reannotation and extended community resources for the genome of the non-seed plant Physcomitrella patens provide insights into the evolution of plant gene structures and functions. BMC Genomics *14*, 498.

〔Review article〕

- Sparks, E.E., Imai, A., Hasebe, M., and Benfey, P.N. (2013). Developmental regulation and *de novo* formation of stem cells in plants. In Stem Cells: From Basic Research to Therapy, F. Calegari, and C. Waskow, eds. (Science Publishers).