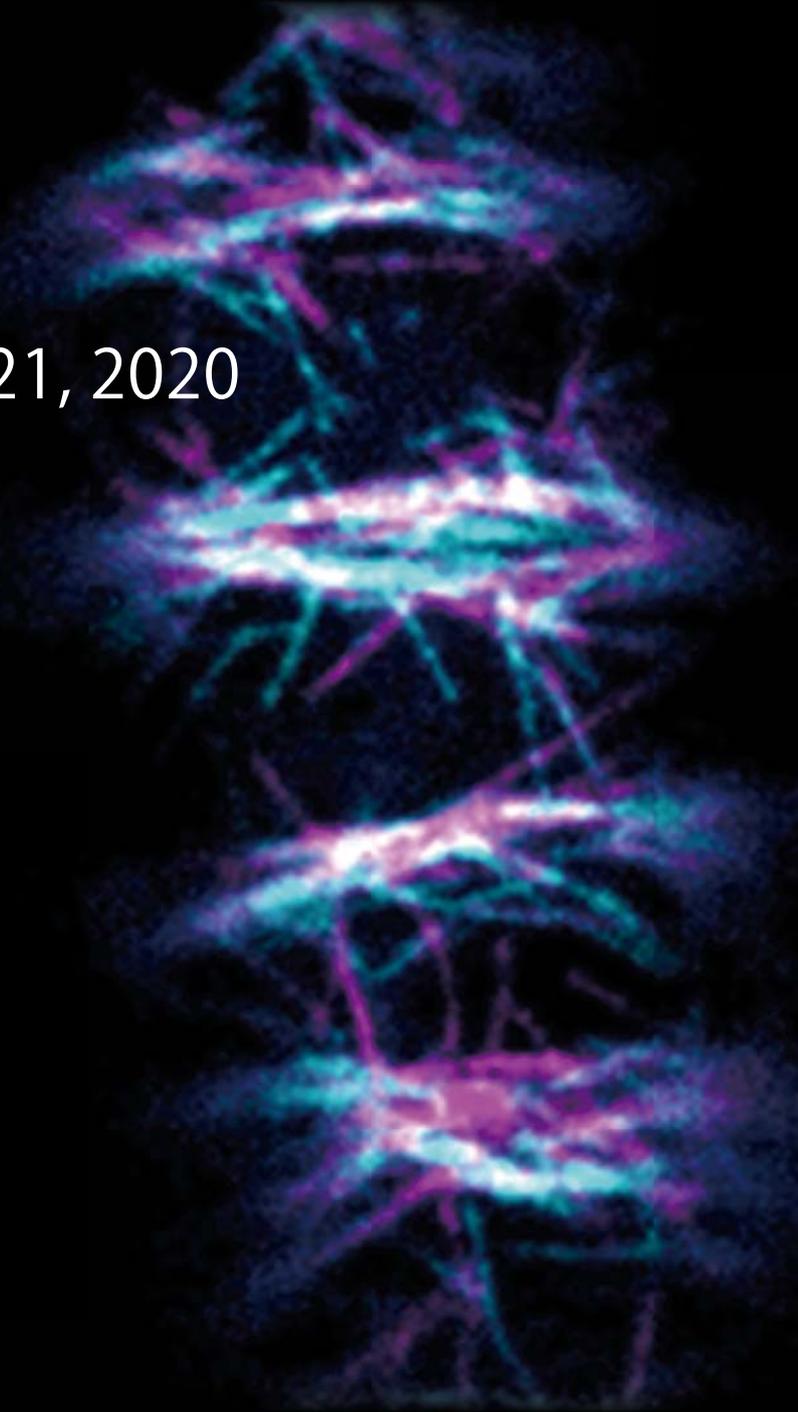


International Webinar Series

FROM CELLULAR DYNAMICS TO MORPHOLOGY

Nov 27 - Dec 21, 2020



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Fri 27. Nov. 17:00-19:00 JST (UTC+9)

Chair: Keiji Nakajima (NAIST)

The last step is the hardest – lignin and suberin formation in the cell wall

Niko Geldner (University of Lausanne)

Chair: Motomu Endo (NAIST)

Mechanisms of auxin-regulated lateral root formation

Hidehiro Fukaki (Kobe University)

Tue 8. Dec. 10:00-12:00 JST (UTC+9)

Chair: Hirokazu Tsukaya (Univ Tokyo)

Orienting asymmetric divisions in the Arabidopsis epidermis

Dominique Bergmann (Stanford University)

Chair: Takashi Ueda (NIBB)

Spatiotemporal dynamics of axis formation in Arabidopsis embryos

Minako Ueda (Tohoku University)

Fri 11. Dec. 17:00-19:00 JST (UTC+9)

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Human time vs. Mouse time with recapitulated systems

Miki Ebisuya (EMBL Barcelona)

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Actual time-series of single-cell transcriptomics reveals circadian clocks as key regulators of cell differentiation in Arabidopsis

Motomu Endo (NARA Institute of Science and Technology)

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Chair: Takayuki Kohchi (Kyoto Univ)

Convergence of cell polarity stems across multicellular kingdoms

Dolf Weijers (Wageningen University)

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Molecular basis of cell wall patterning in xylem vessels

Yoshihisa Oda (National Institute of Genetics)

Thu 17. Dec. 10:00-12:00 JST (UTC+9)

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Painting chromosomes at the nanoscale: a preview into the internal genome organization

Hiroshi Sasaki (Harvard University)

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How plants acquired new organelles -Lessons from Marchantia

Takashi Ueda (National Institute for Basic Biology)

Mon 21. Dec 17:00-19:00 JST (UTC+9)

Chair: Hidehiro Fukaki (Kobe Univ)

Evolution and functional regulation of plant glutamate receptors

José Feijó (University of Maryland)

Chair: Yoshihisa Oda (NIG)

Regulation of sexual reproduction and sex differentiation in gametophyte generation

Takayuki Kohchi (Kyoto University)

Nov. 27 (Fri) 17:00-19:00 JST; 9:00-11:00 CET

The last step is the hardest - lignin and suberin formation in the cell wall

Niko Geldner

University of Lausanne



Lignin and suberin are omnipresent polymers, used by humans since the dawn of civilisation. They are the defining components of wood and cork, but the occurrence and importance of these two polymers is much more widespread and diverse. This is especially evident for the overall little amount of lignin and suberin deposited in the root endodermis, where absence of lignified Casparian strips or suberised secondary cell walls has a profound impact on root permeability and stress resistance. Beyond its direct physiological importance, the endodermis is therefore an interesting model system to study the cell biology of lignification and suberisation. A protracted problem in lignin and suberin research have been the crucial last steps of monomer delivery to, and polymerisation within, the cell wall. Here, I will report on our efforts to generate high-order CRISPR/Cas9-induced mutants of suberin and lignin biosynthetic enzymes, which provide strong evidence for the function of GDSL lipases and peroxidases in suberin and lignin polymerisation, respectively. I will also describe overlooked subcellular structures that are strongly associated with suberin deposition in the endodermis and could be involved in delivery of suberin precursor to the cell walls.

Nov. 27 (Fri) 17:00-19:00 JST; 9:00-11:00 CET

Mechanisms of auxin-regulated lateral root formation

Hidehiro Fukaki

Kobe University



In vascular plants, lateral root (LR) formation contribute to the establishment of the root system architecture. In most eudicot plants, LR formation is initiated by asymmetric division of LR founder cells in the xylem pole of the existing roots, followed by coordinated cell proliferation and differentiation for patterning new LR primordia. The sequential developmental processes of LR formation are triggered by a localized auxin response. In *Arabidopsis*, LATERAL ORGAN BOUNDARIES-DOMAIN 16 (LBD16), an auxin-inducible transcription factor, is one of the key regulators linking auxin response to LR initiation. We have identified the LBD16-regulated genes that positively or negatively regulate LR formation. I will discuss recent results on the mechanisms of auxin/LBD16-regulated LR initiation and lateral inhibition of LR founder cell formation.

Dec. 8 (Tue) 10:00-12:00 JST; 7 Mon 17:00-19:00 PST

Orienting asymmetric divisions in the Arabidopsis epidermis

Dominique Bergmann

Stanford University



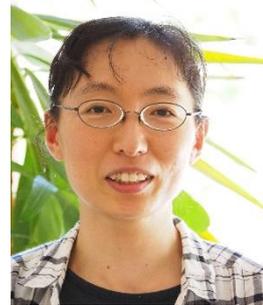
The creation and organization of diverse tissues often relies on asymmetric cell divisions. These divisions that create cells of different sizes and compositions can also be oriented relative to other tissue landmarks. Using the Arabidopsis stomatal lineage, in which multipotent progenitor cells undergo iterative rounds of asymmetric stem-cell like divisions, we have delved into how subcellular reorganization of the cytoskeleton, guided by the polarity protein BASL, leads to oriented cell divisions. Through genetic manipulations and quantitative imaging, we show how BASL acts with the actin and microtubule cytoskeletons before and after cell divisions to ensure the coordination of division plane placement, the inheritance of polarity factors, and stem-cell proliferative capacity.

Dec. 8 (Tue) 10:00-12:00 JST; 7 Mon 17:00-19:00 PST

Spatiotemporal dynamics of axis formation in *Arabidopsis* embryos

Minako Ueda

Tohoku University



Most flowering plants develop from a single-celled zygote. In *Arabidopsis thaliana*, the apical-basal body axis is initiated by asymmetric cell division of the polarized zygote, which possesses the nucleus at the apical cell tip and large vacuoles at the basal end. Despite the obvious asymmetry, the real-time dynamics of the zygote polarization steps was poorly understood. By combining live-cell imaging system of the zygote with various fluorescent reporters and specific inhibitors, we assessed the spatiotemporal dynamics of intracellular structures during zygote polarization. For example, we recently found that the mitochondria associated with the longitudinal array of actin filaments and were polarly distributed along the apical-basal axis. The mitochondria were then temporally fragmented during zygotic division, resulting in unequal segregation into two daughter cells. Based on these results, we would like to discuss various spatiotemporal regulations underlying plant axis formation.

Dec. 11 (Fri) 17:00-19:00 JST; 9:00-11:00 CET

Human time vs. Mouse time with recapitulated systems

Miki Ebisuya

EMBL Barcelona



Different animal species have different tempos of development: larger species tend to grow more slowly than smaller species. My group has been trying to understand the molecular basis of this interspecies difference in developmental time, using the segmentation clock as a model system.

The segmentation clock is the oscillatory gene expressions that regulate the timing of body segment formation during early embryogenesis. We have recently succeeded in recapitulating the segmentation clock from both human and mouse pluripotent stem cells, detecting oscillations and traveling waves in vitro. Interestingly, the oscillation period of human segmentation clock was 5-6 hours while that of mouse was 2-3 hours. Taking advantage of our in vitro system and simple mathematical models, we have been comparing the genome sequences and molecular processes of the segmentation clock between human and mouse to explain the interspecies difference in the oscillation period.

Refs.

- Matsuda et al., "Species-specific segmentation clock periods are due to differential biochemical reaction speeds", *Science*, 369 (2020)
- Matsuda et al., "Recapitulating the human segmentation clock with pluripotent stem cells", *Nature*, 580 (2020)

Dec. 11 (Fri) 17:00-19:00 JST; 9:00-11:00 CET

Actual time-series of single-cell transcriptomics reveals circadian clocks as key regulators of cell differentiation in Arabidopsis



Motomu Endo

NARA Institute of Science and Technology

The basis of toti/pluripotency is elaborate regulation of cell-cycle progression and cell-fate determination. Circadian clocks are involved in this process, but the underlying mechanisms have not been studied due to technical limitations. In particular, there is a lack of research on the universality of cell differentiation mechanisms in multicellular organisms using plants with high cell-fate plasticity. Here, exploiting in vivo single-cell RNA sequencing and a new actual time reconstitution method, PeakMach, we analyzed actual time-series of cell reprogramming and differentiation processes at single-cell resolution, and found that the circadian clock modulates cell differentiation via BES1-mediated GSK3 signaling, which has a β -catenin-like function in Arabidopsis. In this pathway, the clock gene LUX in meristematic stem cells directly targets the CYCD and RETINOBLASTOMA-RELATED (RBR) genes, which are commonly involved in cell-cycle progression and cell-fate determination in plants and animals. In addition, the rhythmicity of the circadian clock was associated with cell state, and the establishment of the circadian rhythm preceded cell differentiation. Thus, our study not only reveals the involvement of the circadian clock in the differentiation of plant stem cells but also demonstrates functionally analogous features in the regulatory system of cell differentiation across species.

Dec. 15 (Tue) 17:00-19:00 JST; 9:00-11:00 CET

Convergence of cell polarity stems across multicellular kingdoms

Dolf Weijers

Wageningen University



Cells in multicellular organisms organise along body and tissue axes. Cellular processes, such as division plane orientation, must be aligned with these polarity axes to generate functional 3-dimensional morphology, particularly in plants, where cell walls prevent cell migration. While some polarly localized plant proteins are known, molecular mechanisms of polarity establishment or its translation to division orientation are elusive, in part because regulators in animals and fungi appear to be missing from plant genomes. Cell polarity is first established in the embryo, but this has long been an intractable experimental model. In the past years, genetic and imaging tools have been developed that allow using the early *Arabidopsis* embryo as a model for understanding the mechanisms that drive cell polarity and oriented cell divisions, I will present our recent work, focusing on the identification of a novel, deeply conserved polarity system.

Dec. 15 (Tue) 17:00-19:00 JST; 9:00-11:00 CET

Molecular basis of cell wall patterning in xylem vessels

Yoshihisa Oda

National Institute of Genetics



Polarity establishment is essential for cellular development, behavior, and function. Yet, how multiple polarity is initiated and organized in a cellular space is poorly understood. Plant xylem vessel cells deposit cell walls in beautiful patterns such as annular, spiral, reticulate, and pitted patterns, directed by multiple domains of microtubule array. We revealed that the small G protein ROP is locally activated on the plasma membrane, disassembles microtubules, and thereby directs formation of pitted cell walls. The local activation of ROP is cell-autonomously triggered through the action of ROP regulators. Microtubules laterally confine the localization of ROP along the plasma membrane to fine-tune the shape of cell wall pits. We also found that ROP locally assembles actin microfilaments to promote cell wall deposition specifically at the edge of cell wall pits. We will discuss how multiple polarity is generated and transformed into the distinct cell wall pattern in xylem vessels.

Dec. 17 (Thu) 10:00-12:00 JST; 16 Wed 20:00-22:00 EST

Painting chromosomes at the nanoscale: a preview into the internal genome organization

Hiroshi Sasaki

Harvard University



Eukaryotic cells package a genome with a linear length of meters into a nucleus that is a mere 10 μm in diameter. Despite this dramatic difference in length scales, the organization of DNA within the nucleus is non-random and plays an important role in many nuclear processes such as transcription, duplication, and repair. The recent introduction of super-resolution chromatin imaging methods, in combination with fluorescence in situ hybridization (FISH), has transformed our view on basic organizational features of the genome in individual cells. However, our mechanistic understanding of how the genome works is far from complete due to technical limitations, as it remains challenging to image chromatin on the order of tens of nanometers, a scale that could reveal rich structural detail about the genome organization. We introduce a robust and multiplexed super-resolution FISH approach leveraging our super-resolution imaging method called 'DNA-PAINT' and signal-amplification method 'SABER' for quantitative chromatin imaging at a spatial resolution close to the nucleosome scale with a simple and easy-to-implement optical set-up. We have applied our approach to sampling the ultrastructure of the human X chromosomes. Contrary to the prevailing view that the inactive X chromosomes consist of facultative heterochromatin, we have found no difference in the apparent compaction level between the active and inactive X chromosomes. Our approach will enable researchers to perform a molecular-level analysis of nuclear organization and architecture in individual cells that we expect to be broadly applicable to a wide range of biological questions.

Dec. 17 (Thu) 10:00-12:00 JST; 16 Wed 20:00-22:00 EST

How plants acquired new organelles - Lessons from *Marchantia*

Takashi Ueda

National Institute of Basic Biology



"The membrane trafficking system responsible for transporting proteins, lipids, and polysaccharides plays pivotal roles in various plant functions including development, defense responses, intercellular communication, and cell wall biogenesis. This system involves evolutionarily conserved machinery components such as SNARE proteins, which execute membrane fusion between transport vesicles and destination membranes. The number of genes for SNARE proteins has been increased during land plant evolution, which should be associated with diversification of membrane trafficking pathways and/or acquisition of new organelles. For insights into diversification of the membrane trafficking system during land plant evolution, we are conducting comparative analyses of membrane trafficking pathways between *Arabidopsis thaliana* and *Marchantia polymorpha*. We systematically identified SNARE proteins in *M. polymorpha*, whose expression patterns, subcellular localizations, and molecular functions were investigated. We discovered that a SNARE member belonging to the SYP1 family, which generally mediates membrane fusion at the plasma membrane (PM), localizes to the membrane of a liverwort-specific organelle, the oil body, suggesting that the oil body membrane shares similar properties with the PM. We also found that the luminal space of the oil body is topologically equivalent to the extracellular space. These lines of evidence indicated that the oil body is formed by redirection of the secretory pathway from outward to inward the cell. Furthermore, we identified a master regulator of oil body formation, under which expression of a set of oil body-related factors including the SYP1 member are regulated. We propose that the direction of the secretory pathway is periodically reoriented depending on transition of cellular states in oil body cells, as observed in cell plate formation in mitotic cells.

Dec. 21 (Mon) 17:00-19:00 JST; 8:00-10:00 WET

Evolution and functional regulation of plant glutamate receptors

José Feijó

University of Maryland



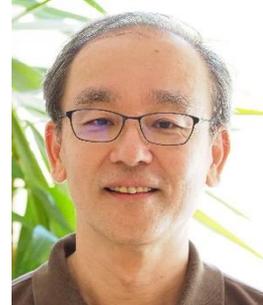
Ionotropic glutamate receptors (iGluRs) in the animal Central Nervous System are vital for high fidelity synaptic transmission. Glutamate, the principal neurotransmitter, binds to an extracellular ligand-binding domain to open the transmembrane ion channel. This action drives a depolarization of the postsynaptic membrane and promotes Ca^{2+} transport. GLutamate Receptor-like channels (GLRs) are the plant homologs of iGluRs and are also associated with electrical and Ca^{2+} signaling to participate in essential mechanisms, including developmental processes, responses to (a)biotic stress, sexual reproduction, and systemic signaling. The evolutionary relationship between iGluRs and GLRs was recently verified, finding structural homology in the extracellular ligand-binding domain (LBD) — yet the structure-function relationships of GLRs are only emerging. I'll present and discuss data on the characterization of GLRs expressed in moss protonema and heterologous systems, demonstrating the requirements for functional ion channel operation and the channel properties that distinguish GLRs from iGluRs. With a structurally conserved ligand-binding domain, albeit conferring different ligand-gating properties, we show the GLR ion channel pore plays a direct role in ion channel gating and ion selectivity. We further address the conservation of these properties in Arabidopsis GLRs, and address the vexing problem created by the diversification and strong expansion of GLRs in flowering plants. These results aim to enlighten the molecular evolution of plant glutamate receptors shaping the ion channel properties that ultimately conserve a role in Ca^{2+} and electrical signaling and have strong implications in our current interpretation and understanding of GLRs biological functions.

Dec. 21 (Mon) 17:00-19:00 JST; 8:00-10:00 WET

Regulation of sexual reproduction and sex differentiation in gametophyte generation

Takayuki Kohchi

Kyoto University



Bryophytes occupy a basal position in the monophyletic evolution of land plants and have a life cycle in which the gametophyte generation dominates over the sporophyte generation. We recently showed that BONOBO, a member of the bHLH transcription factors regulates reproductive development including germ cell differentiation in the liverwort *Marchantia polymorpha* and generative cell specification in *Arabidopsis*. We also reported that MpFGMYB shares functions in female gametophyte development with the ortholog in *Arabidopsis*. As above, many orthologs of angiosperm transcription factors play key roles in bryophyte sexual reproduction processes, including germline cell specification and differentiation, gamete formation, and haploid-to-diploid transition, suggesting that the land plant reproductive systems have conserved the core molecular mechanisms despite significant differences in morphology. In this seminar, we would also like to discuss unique sex determination system mediated by cis-acting lncRNA and 'Feminizer' encoded in the sex chromosome in *M. polymorpha*.