



Periodicity and  
its Modulation in Plants  
植物の周期と変調

International Webinar Series

# FROM CELLULAR DYNAMICS TO MORPHOLOGY III

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“Periodicity and its Modulation in Plants”



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Dynamic Nutrient Signaling Networks in Plants

Kun-Hsiang Liu<sup>1,2</sup>, Ruiqiang Ye<sup>1,3</sup>, Jen Sheen<sup>1</sup> (<sup>1</sup>Massachusetts General Hospital/Harvard Medical School, USA; <sup>2</sup> Northwest Agriculture & Forestry University, China; <sup>3</sup>CAS Center for Excellence in Molecular Plant Sciences, China)

[Session V] Tue 6. Dec. 17:00-19:30 JST (UTC+9)

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Placing new walls: Molecular mechanisms of division plane control in plants

Katharina Bürstenbinder (Leibniz Institute of Plant Biochemistry, Germany)

Delivering intracellular materials: Roles and mechanisms of microtubule-based transport in plants

Gohta Goshima (Nagoya University, Japan)

[Session VI] Mon 19. Dec 17:00-19:30 JST (UTC+9)

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Spatial control of plant steroid receptors in plant adaption to climate stress

Ana I. Caño-Delgado (Center for Research in Agricultural Genomics, Spain)

*Monophyllaea* shoot system is composed of a single, indeterminate cotyledon with no additional organ

Hirokazu Tsukaya (The University of Tokyo, Japan)

[Session I] Tue 22. Nov. 17:00-19:30 JST (UTC+9)



## **Regulation of cellulose synthesis in land plants**

***Staffan Persson***

*University of Copenhagen, Denmark*

All plant cells are surrounded by a cell wall that provides protection, neighbor adhesion and the basis of morphology of plant cells. Cellulose is also a major raw material for many industries, including the paper, textile and fuel industries. Plant cell walls are mainly built by polysaccharides of which cellulose typically constitute the major component. Cellulose is made at the plasma membrane by large CELLULOSE SYNTHASE (CESA) protein complexes that move forward in the membrane due to the immobilization of the nascent cellulose chains in the cell wall. The direction of the movement is steered by underlying cortical microtubules. My group has outlined many aspects of cellulose synthesis and the principles of how the CESA complex is guided by microtubules. In this talk, I will highlight recent progress in our knowledge of how the cellulose synthesis is controlled on different levels, including transcriptional and post-translational controls.

[Session I] Tue 22. Nov. 17:00-19:30 JST (UTC+9)



**Understanding self-organizing pattern on plant cell wall by mathematical analyses**

***Atsushi Mochizuki***

*Kyoto University, Japan*

In plant xylem vessels, thin cell wall regions called “cell wall pits” appear periodically. A series of studies by Oda et al. has revealed that ROP, a Rho-GTPase in plants, plays an important role in the periodic patterns of cell wall pits. On the plasma membrane surface, active ROP forms a two-dimensional periodic distribution that determines the region corresponding to future cell wall pits. We hypothesized that the periodic patterns of ROPs are generated by self-organization due to diffusional instability (Turing instability), and constructed a reaction-diffusion model that takes into account the state-transition reaction of ROPs and their movement across the plasma membrane. Three possible models based on biological facts are developed: (a) a basic model including only the state transition of ROP, (b) a negative feedback model via conservation of the total amount of GEF and GAP, and (c) a positive feedback model to the GEF binding reaction. Model (c) is based on the possibility of GEFs forming dimers. We analyzed these models mathematically to determine the conditions under which the uniform distribution of ROPs is instabilized and a periodic pattern is generated. As a result, we found that neither (a) the basic model nor (b) the negative feedback model can ever form a periodic pattern, and that only (c) the positive feedback model can generate a periodic pattern. In other words, the positive feedback to the GEF binding reaction is essential for the formation of the periodic patterns of cell wall pits. Numerical simulations with various parameter values were performed to quantitatively determine the conditions for the formation of the periodic patterns.

[Session II] Mon 28. Nov. 17:00-19:30 JST (UTC+9)



## **Auxin signaling: more than we have ever imagined**

***Jiří Friml***

*Institute of Science and Technology, Austria*

The plant hormone auxin is a versatile intercellular signal influencing virtually all aspects of plant life. It has a unique ability to be directionally transported within tissues forming local auxin maxima or gradients that are central to many developmental processes mediated by auxin. One of the key roles of auxin is adaptation of plant growth to gravity, where shoots bend up and roots down. This paradox is based on opposite responses of these organs to the phytohormone auxin, which promotes cell expansion in shoots, while inhibiting it in roots via an unclear signalling pathway and yet unknown downstream cellular mechanism

The well-established canonical auxin signalling involving the TIR1/AFB auxin receptors, Aux/IAA repressors and ARF transcription factors acts in nucleus and mediates gene transcription. However, auxin also triggers cellular responses within seconds or minutes, too fast to rely on transcription. Part of the rapid responses is mediated by the non-transcriptional branch of the TIR1/AFB signalling, but others involve a yet completely unknown mechanism.

Here I will present new and surprising insights into the mechanism of auxin signalling including an ultrafast auxin-triggered protein phosphorylation response and previously unsuspected aspects of TIR1/AFB auxin perception and downstream signalling.

**Keywords:** Auxin, Arabidopsis, root gravitropism, TIR1/AFB signalling

**Acknowledgements:** I gratefully acknowledge all present and past members of Friml group and our excellent collaborators.

[Session II] Mon 28. Nov. 17:00-19:30 JST (UTC+9)



**Cell polarity establishment and maintenance by NPH3-like protein-mediated PIN cluster formation in land plants**

***Satoshi Naramoto***

*Hokkaido University, Japan*

Cell polarity reflected by asymmetric distribution of proteins at the plasma membrane (PM) is essential for various cellular processes. PIN-FORMED (PIN) proteins are prominent polarly localized PM proteins in seed plants and are proposed to undergo endocytic recycling to establish and maintain polar localization of PIN proteins. The findings obtained so far provide some conceptual framework of how PIN polarity is established and maintained. However, there are still many open questions on the polar localization of PIN proteins. One of the main unresolved issues is the dynamics and behavior of PIN proteins at PMs. Here we showed that at PMs PIN2 proteins form clusters that are organized into linear arrays parallel to but non-overlapping with cortical microtubule and are also independent of endocytic machinery. We also identified that NPH3-like protein MAB4 and its homolog MAB4/ENP-like (MEL)s induce PIN2 clustering, which in turn inhibit lateral diffusion and endocytosis of PIN2 proteins to maintain polar localization of the PIN proteins. Furthermore, we determined that PIN clusters are structurally flexible protein complexes that are also present in bryophytes, suggesting that PIN clusters are conserved across land plants. Our findings suggest a crucial role for PIN clustering in its polar localization, and identify that plants have evolved a unique mechanism for protein stabilization at specific PM regions as a basis for cell polarity regulation.

[Session II] Mon 28. Nov. 17:00-19:30 JST (UTC+9)



**Low-auxin responsiveness is key to stem cell regulation in the liverwort *Marchantia polymorpha***

***Ryuichi Nishihama***

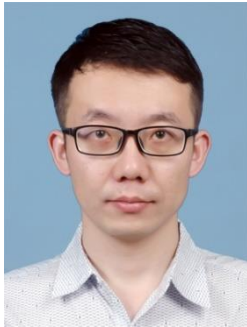
*Tokyo University of Science, Japan*

Development of all land plants is characterized with apical growth, by which new tissues and organs are formed from stem cells residing in the apical meristem. Plants enlarge their bodies by branching and also create new plants by regeneration, both of which involve amplification of stem cells. The major phytohormone auxin is involved in these processes. Recent studies revealed that the mechanism for auxin-mediated transcriptional regulation is highly conserved throughout land plants. However, our knowledge on the roles of auxin in stem cell regulation in bryophytes is still limited. We address this issue with the liverwort *Marchantia polymorpha*.

When a thallus of *M. polymorpha* is bisected into the apical and basal halves, a new thallus regenerates only from the cut surface on the basal half. Therefore, auxin produced at the meristem has been thought to inhibit regeneration. We show that the endogenous indole-3-acetic acid level transiently and rapidly decreases at the cut site of decapitated explants, which then triggers expression of the cellular reprogramming factor *LOW-AUXIN RESPONSIVE* (*MpLAXR*) (Ishida et al., 2022). Even in intact plants, *MpLAXR* can be up-regulated when auxin signaling is manipulated to diminish. Knockout of the sole auxin receptor for the canonical nuclear pathway, *MpTIR1*, causes cell clump formation. Transcriptome analysis revealed that the transcriptional response to auxin is largely impaired in the *Mptir1* mutant cells and that their transcript profile has a stem cell property with *MpLAXR* up-regulated, consistent with the low-auxin responsiveness of this gene (Suzuki et al., in revision). These findings highlight the key role of low-auxin responsiveness in stem cell regulation. In this talk, we will also present our recent results and discuss gene regulatory networks for this process.



[Session III] Wed 30. Nov. 17:00-19:30 JST (UTC+9)



### **Plant responses to water stress: back to the roots**

***Yang Zhao***

*Shanghai Center for Plant Stress Biology, CAS Center for Excellence in Molecular Plant Sciences, China*

My research aims to decipher the stress-induced stimuli, identify sensors and core signaling components, and illustrate growth regulation mechanisms, ultimately improving plant stress tolerance. We have established scientific models and research systems for osmotic and salt stress signaling. In past years, we discovered the plasma membrane-localized protein OSMO1/BON1 controls early osmotic stress signaling (Current Biology, 2020). We have also found that ABA-activated SnRK2s phosphorylate SWEET sucrose transporters and microtubule-binding protein SP2L, which mediates root growth and halotropism under stressed conditions (Nature Plants, 2022; Developmental Cell, 2022). I will present these discoveries, especially the cellular and molecular mechanisms of root halotropism.

[Session III] Wed 30. Nov. 17:00-19:30 JST (UTC+9)



**Deciphering the time- and space-dependent cellular dynamics of Arabidopsis root cap**

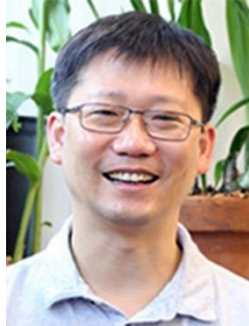
***Keiji Nakajima***

*Nara Institute of Science and Technology, Japan*

The root cap is a multi-layered tissue covering the tip of a plant root and directs root growth with its unique functions such as gravity-sensing and secretion. To maintain its structure and functional integrity, the root cap cells continuously turnover through balanced proliferation of its inner stem cells and dehiscence of the outer mature cells. Such cellular turnover is seen in many animal tissues, but is unique among plant tissues. To dissect genetic and molecular mechanisms underlying the periodic turnover of root cap cells, we developed a motion-tracking confocal microscope system and used it to visualize both cellular and subcellular dynamics of the Arabidopsis root cap, as well as their associated gene expression patterns. By combining omics and genetic approaches, we have started to identify novel mechanisms that spatiotemporally regulate cell separation and organelle rearrangement of the root cap according to time and space.

[Session IV] Mon 19. Dec 17:00-19:30 JST (UTC+9)

## Dynamic Nutrient Signaling Networks in Plants



**Kun-Hsiang Liu** <sup>1,2</sup>



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Nutrient signaling is the most ancient and fundamental mechanism to regulate and sustain life and acts to modulate cellular activities and organismal development by integrating with other intrinsic regulators and environmental cues. In contrast to the previously prevailing notion that nutrients automatically feed into cellular metabolism and growth, nutrient signaling mechanisms are complex for the tailored regulatory networks in diverse cell types, tissues, and organs with specialized physiology, metabolism, and functions. Current models of regulatory networks in plant development have mainly focused on hormone and peptide signaling. However, hormones and growth factors are ineffective in promoting growth without nutrient signaling. To elucidate nutrient-mediated signaling mechanisms in plants, new experimental approaches have been developed to circumvent limits due to gene redundancy and mutant embryo lethality. We have developed targeted functional genomic screens, chemical genetic tools for lethal mutants, genomic profiling analyses, ultrasensitive  $\text{Ca}^{2+}$  ratiometric sensors, and PUP-IT-based proximity tagging screens for identifying new nutrient sensing and signaling components in *Arabidopsis thaliana* as a reference plant. Our efforts have led to a surprising molecular link in broad controls of plant development by epigenomic reprogramming via glucose-driven TOR-FIE-PRC2 signaling. We also discovered that specific  $\text{Ca}^{2+}$  sensor protein kinases (CPKs) and NODULE INCEPTIONLIKE PROTEIN (NLP) transcription factors play pivotal roles in plant nitrate signaling in establishing shoot and root architecture. We have shown that the combinatorial functions of NLP2,4,5,6,7,8,9 control primary nitrate responses that orchestrate the transcriptional network to promote nitrate transport/assimilation, metabolism

reprogramming, and plant development. Our recent findings have identified NLP7 as a previously unrecognized dual nitrate sensor and transcription activator, which enabled the creation of the first genetically encoded split-fluorescent nitrate biosensor to visualize real-time intracellular nitrate dynamics over a broad concentration range in diverse organs in plants.



[Session V] Tue 6. Dec. 17:00-19:30 JST (UTC+9)



**Placing new walls: Molecular mechanisms of division plane control in plants**

***Katharina Bürstenbinder***

*Leibniz Institute of Plant Biochemistry, Germany*

Spatio-temporal control of cell division is crucial for morphogenesis of multicellular organisms, and most particularly in plants, where cells are physically glued by cell walls that prevent cell migration. To divide in the presence of rigid walls, cell division has undergone a switch from a cleavage-like mode, typically found in simple algae and animals, to an inside-out mechanisms, in which new cell walls are inserted at the cell center and expand centrifugally to fuse with the maternal cell wall. Two plant-specific cytoskeleton arrays, the preprophase band (PPB) and phragmoplast play essential roles in division plane positioning and cell plate formation. The assembly and dynamics of these mitotic microtubule arrays are controlled by multiple functionally distinct classes of microtubule-associated proteins (MAPs). Although functions of individual MAPs have been characterized in detail, it still is largely elusive how networks of MAPs act in concert to coordinate the plant cytoskeleton. Here, I will present and discuss how a class of scaffold-like MAPs, termed IQ67 domain (IQD) proteins facilitates the assembly of MAPs and other interacting proteins at microtubules and membranes to coordinate PPB formation, division plane set up, and cell plate positioning. Together, our analyses provide insights into molecular mechanisms of division plane control, and principles and regulation of macromolecular complex assemblies that are essential for plant growth and development.

[Session V] Tue 6. Dec. 17:00-19:30 JST (UTC+9)



**Delivering intracellular materials: Roles and mechanisms of microtubule-based transport in plants**

***Gohta Goshima***

*Nagoya University, Japan*

Over the past decade, our laboratory has been delving deep into two long-standing questions in plant microtubule biology: how do plant cells assemble the mitotic spindle in the absence of centrosomes, and how do they execute microtubule-based transport without key molecular motors found in animal cells? I will present what we have learnt on these issues, in particular the identity of the long sought-after molecular motors of microtubule-based transport in plants.

[Session VI] Mon 19. Dec 17:00-19:30 JST (UTC+9)



**Spatial control of plant steroid receptors in plant adaption to climate stress**

***Ana I. Caño-Delgado***

*Center for Research in Agricultural Genomics, Spain*

Abstract

[Session VI] Mon 19. Dec 17:00-19:30 JST (UTC+9)



***Monophyllaea* shoot system is composed of a single, indeterminate cotyledon with no additional organ**

***Hirokazu Tsukaya***

*The University of Tokyo, Japan*

The genus *Monophyllaea* belongs to the Gesneriaceae of Eudicot. Members of *Monophyllaea* are distributed in the limestone regions of the tropics of Southeastern Asian and are known as 'one-leaf plants'. This is because they have only one leaf that grows indeterminately during the vegetative phase. This indeterminate leaf originates from one of the two cotyledons that spread evenly at germination. Shortly after germination, the two cotyledons compete with each other and only one leaf survives to grow. Our surgical experiment showed that fate determination occurs after germination. We recently revealed that skewed auxin concentration between the two cotyledons and subsequent cytokinin levels are involved in fate determination.

Instead one of cotyledons acquires indeterminacy of growth, no shoot apical meristem (SAM) activity is recognized between these cotyledons during the vegetative phase. This queer shoot system is called 'phyllomorph'. RNAseq analysis combined with whole mount *in situ* hybridization analysis, we found that the SAM regulator *STM* and a leaf meristem regulator *AN3*, both known in common plant species, are co-expressed in the basal region of the indeterminately growing cotyledon. This fact suggests that phyllomorph, the indeterminate leaf, is a kind of a chimera of shoot and leaf. Based on our recent additional data on the other SAM- and leaf meristem-related genes, we discuss possible regulatory mechanisms of this unique phyllomorph system.