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Meristem Biology Flourishes Under Mt. Tai

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The green and lush Mt. Tai provided an appropriate backdrop for the First International Conference on Plant Meristem Biology held on June 9 and 10, 2016. Proposed and organized by Xian Sheng Zhang (Shandong Agricultural University, China), Jiayang Li (Institute of Genetics & Developmental Biology, China), Elliot Meyerowitz (California Institute of Technology, USA), and Yuling Jiao (Institute of Genetics & Developmental Biology, China), the conference was hosted by Shandong Agricultural University and sponsored by the Department of Education, Shandong Province, China. The first meeting of its kind, it brought about 300 attendees from nine countries together for discussions on molecular mechanisms underlying shoot, root, cambium, and adventitious meristems.

SHOOT APICAL MERISTEM

Shoot apical meristems (SAM) are responsible for the generation of all aerial organs. They comprise dividing cells and are highly organized, both histologically and genetically. In the past, the power of genetics has proven successful to unravel key factors involved in SAM function. In particular, it is now well established that the population of stem cells (expressing the CLV3 peptide) is maintained through the positive action of the homeodomain protein WUS (expressed in the organizing center) on *CLV3* expression. In turn, CLV3 represses *WUS* expression through the CLV1/CLV2-CRN pathway.

In this conference, genetics was still strong, demonstrating that despite the accumulation of knowledge, we are far from having an integrated view of the SAM gene network. Some chromatin regulators were put forward. For instance, **Ralf Müller-Xing** (Northeast Forestry University) identified a new mutant allele of *CLF* through a suppressor screen of *p35S::CLV3* expressing lines (and displaying a *wus*-like phenotype). The observed suppression of stem cell loss seems partially independent of *WUS*, indicating stem cell regulation by PcG beside the CLV3-WUS or the AG-WUS feedback loops. Physical interactions between SAM factors were revealed. In particular, **Ying Hua Su** (Shandong Agricultural University) proposed that STM acts as a co-factor of WUS, STM was also found to directly activate the expression of *CLV3*, suggesting that STM and WUS function synergistically in the regulation of stem cell activity. The activity of transcription factors was further investigated through the analysis of binding sites in promoters. For instance, **Venu Reddy** (University of California, Riverside) identified *cis* elements in the *CLV3* promoter, with different affinity for WUS, depending on whether WUS is a monomer or a dimer. The activity of these genetic regulators is orchestrated by hormones, also reflecting in part environmental conditions. In that respect, **Jan Lohmann** (Heidelberg University) showed that *WUS* is not expressed in germinating seedlings in the dark. Its expression is switched on in the presence of light. COP1, but not HY5, was involved in that response. Knowing that CKX5 and CKX6 actively degrade cytokinins in the dark, *WUS* was found to be induced in the dark in a *ckx5 ckx6* background, providing a model in which light conditions control cytokinin levels to start the CLV3-WUS loop and thus meristem activity.

In addition to cytokinins, auxin has also been shown to play a key role in organ initiation. Auxin also accumulates in the CZ of the meristem, but its contribution to meristem function has remained unclear. **Zhong Zhao** (University of Science and Technology of China) found that the auxin response factor ARF5/MP is present in the CZ, and directly represses a novel factor called MTA1. Interestingly, MTA1 induces *CLV3* expression, thus demonstrating that auxin, via MP and MTA1, is involved in stem cell maintenance in the CZ. Last, **Zhi Juan Cheng** (Shandong Agricultural University) showed that ARF3 not only represses *IPT5* expression (and thus cytokinin accumulation) but also *WUS* expression. This action is mediated in a complex containing disordered protein (DP1/2) that binds ARF3 and ARF4.

Beyond the gene network analysis, the impact of these regulators in the end is on growth and patterning. As **Elliot Meyerowitz** (California Institute of Technology) emphasized, this involves major changes in cell wall composition and regulation. In particular, the cellulose synthase-like *CSLD5* gene appears as a central regulator of meristem size and maintenance. Analysis of cell wall composition also reveals qualitative differences between meristem and other tissues. Interestingly, modifying the cell wall composition can in turn modify the activity of meristem regulators, like the polarity of PIN1 proteins, notably by affecting tension patterns in the SAM. More generally, mechanical stress

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contributes to meristem functions. **Olivier Hamant** (ENS de Lyon) showed that new cell division planes align along maximal tensile stress directions, whether stress is prescribed by cell shape or by differential growth or tissue shape. Mechanical stress also affects the expression of certain genes, like *STM*, the promoter activity of which is promoted in the SAM boundary, a domain under high and directional stress.

AXILLARY MERISTEM AND FLORAL MERISTEM

A key determinant of plant architecture is the axillary meristem (AM), which forms in the axils of leaves. AM develops into the axillary bud, which can grow out to form branches in response to developmental and environmental signals. Furthermore, floral meristem (FM) is considered a specialized AM formed after floral transition. Whereas axillary buds have the same indeterminate growth potential as the shoot apex, FM is only transiently maintained to give rise to floral organs with fixed numbers.

AMs initiate from the leaf axils, which are boundaries separating the leaf from the stem. **Klaus Theres** (Max Plank Institute for Plant Breeding Research) emphasized how leaf axil boundaries and leaflet boundaries, which separate leaflets in plants with compound leaves, such as tomato, share a similar regulatory mechanism and developmental potential. Notably, new meristems can also initiate from distal leaflet boundary in tomato. He showed how several transcription factor-encoding genes commonly affect the leaf axil boundary and leaflet boundary formation. In addition to transcription factors, hormones can also regulate boundary and AM initiation. **Quan Wang** (Agricultural Genomics Institute at Shenzhen) showed boundary auxin minimum conditions competence for AM formation from leaf axil boundary in *Arabidopsis* and tomato. Both auxin influx and efflux are utilized to move auxin away from the boundary to establish the leaf axil auxin minimum. An open question is which cells are competent to form AMs. **Yuling Jiao** (Institute of Genetics & Developmental Biology) used cell lineage tracking and ablation experiments to show that a group of *STM*-expressing cells are necessary for AM initiation. He further found that, in addition to maintained weak *STM* expression, REV-promoted upregulation of *STM* is required for AM initiation. Finally, cytokinin accumulation in the leaf axil *de novo* activates *WUS* expression to complete AM initiation, in which B-type ARR transcription factors directly bind to the *WUS* promoter regions.

Shoot branching significantly affects crop yield because it determines tiller number (and thus spike number) and spike complexity (and thus seed number). Using rice as a model, **Jiayang Li** (Institute of Genetics & Developmental Biology) showed that two transcription factors, MOC1 and MOC3 (a rice homolog of WUS), regulate AM initiation, and a MOC1-MOC3 heterodimer is formed to exert their molecular functions. Furthermore, he reported that MOC1 may form a heterodimer with gibberellin signaling pathway components, suggesting a link between shoot branching and plant height. Similar to AM initiation, bud dormancy is also negatively regulated by auxin. **Yonghong Wang** (Institute of Genetics & Developmental Biology) showed that mutations of *MKK7*, a gene of the mitogen-activated protein kinase cascade, in *Arabidopsis* results in a bushy phenotype. Biochemical analysis indicated that MKK7 specifically phosphorylate a downstream target to control polar auxin transport, consistent with the shoot branching phenotype. Rice panicle branching directly affects yield. **Xiangdong Fu** (Institute of Genetics & Developmental Biology) found that a major QTL corresponds to *DEP1*, a gene encoding an atypical G protein beta subunit. A dominant *dep1* allele exhibits not only increased panicle branching but also increased nitrogen-use efficiency. On the other hand, a DEP1-interacting protein regulates grain size. Thus, modulating *DEP1* may simultaneously improve multiple traits.

Although FM shares similarity with AM, FM only maintains transient meristem activity, as in leaves. Investigating the pathway that leads to termination of the FM, **Toshiro Ito** (Nara Institute of Science and Technology) showed that the transcription factor KNUCKLES (KNU) represses *WUS* expression, first by evicting SPLAYED from the *WUS* promoter, and second by recruiting PcG, leading to H3K27me3 and stable silencing of *WUS* expression. In addition, *TORNADO2* (*TRN2*) mediates auxin transport downstream of *CRABS CLAW* (*CRC*). The *knu crc* double mutant shows enhanced FM indeterminacy. While KNU directly represses *WUS*, CRC directly represses *TRN2* transcript, which affects auxin transport in the FM. **Wanqi Liang** (Shanghai Jiao Tong University) presented a new regulator of auxin distribution in rice FM, called ABERRANT FLOWER MERISTEM, which is more highly expressed in floral organ primordia and leads to higher auxin transport toward the FM when mutated.

ROOT APICAL MERISTEM

Root apical meristem (RAM) is initiated by the specification of a single cell, the hypophysis, during embryogenesis in *Arabidopsis*. RAM is responsible for the generation of new root organs during postembryonic development. In *Arabidopsis*, the RAM is an area comprising four mitotically inactive quiescent cells (QC) surrounded by mitotically active stem cells, which controls root growth and development. The simple structure of RAM makes it an ideal system to study plant stem cell activity. Previous investigations have highlighted the critical roles of plant hormones, such as auxin and cytokinin, and key transcription factors, such as AP2 family transcription factor PLTs (PLETHORAs), GRAS family transcription factors SCR (SCARECROW) and SHR (SHORT ROOT), and QC-specifically transcribed WOX5 in the maintenance of RAM.

At this conference, several key results have further deepened our understanding of the molecular mechanisms behind RAM regulation. Using a genome-wide approach, **Joakim Palovaara** (Wageningen University) showed a high-resolution gene expression map of the root stem cell niche. This study suggests a spatiotemporal shift in cell-specific expression relating not only to clonal origin but also to the cellular position in the embryo. Such transcriptional reprogramming was further investigated through local inhibition of auxin response, which is well known to control the initiation and maintenance of RAM. Besides auxin, jasmonate (JA) was also demonstrated by **Chuanyou Li**'s lab (Institute of Genetics & Developmental Biology) to control RAM through MYC2-regulated expression of *PLT1* and *PLT2*. They

also found that JA controls ASA1-dependent auxin biosynthesis and attenuates PIN-dependent auxin transport, providing fine-tuned regulation of local auxin accumulation in the root basal meristem that is essential for lateral root formation.

Alternative splicing is an important mechanism for increasing transcriptome plasticity and proteome diversity in eukaryotes. To understand the role of alternative splicing in root development, **Masashi Yamada** (Duke University) generated comprehensive expression data of almost all root cell types and found a remarkable change in expression of alternative spliced isoforms of a given cell type among different developmental stages. However, there were no significantly different splicing variations among cell types at the same developmental stage. Overexpressing one splicing isoform resulted in premature root development without affecting cell-type differentiation. This study suggests that alternatively splicing has a primary role in regulating root development, even dominating cell-type specification.

In *Arabidopsis* root, QC cells divide at a low frequency, which is essential for the maintenance of RAM. The low division rate is likely contributing to the resistance of DNA damage, which was shown by **Lieven De Veylder** (Ghent University). Using a combination of genetics and biochemistry, they identified a new transcription regulator complex that controls cell division upon loss of stem cells. Remarkably, the co-expression of all complex subunits grants differentiated cells the ability to obtain a QC identity, resulting in ectopic *de novo* establishment of stem cell niches. Reversely, activity of both subunits is required for re-establishment of new stem cell niches upon loss of QC cells after ablation.

Reactive oxygen species (ROS) have been well known to play an important role in the maintenance of stem cell identity in mammals, including humans. However, in plants, the role of ROS in the control of root stem cell identity is not well understood. **Zhaojun Ding** (Shandong University) showed that the *Arabidopsis APP1*, which encodes a P-loop NTPase, was involved in the maintenance of root QC and distal stem cell (DSC) identity through its control of local ROS homeostasis. The disruption of APP1 was accompanied by a reduction in the ROS level, a rise in the rate of cell division in the QC, and promotion of root DSC differentiation. Through chemical treatment, they also found that both high levels of ROS and low levels of ROS induced QC cell division and DSC differentiation, suggesting that an optimum of ROS levels is required for the maintenance of root QC and DSC identity.

It has long been known that WOX5 is specifically transcribed in the QC, and is required to maintain root stem cell niche identity. However, downstream targets of WOX5 were largely unknown. **Limin Pi** (University of Freiburg) showed that WOX5 maintains its stem cell niche through WOX5-TPL/TPR-HDA19 complex-medicated histone deacetylation and repression of the differentiation factor CDF4.

CAMBIUM

During root development, xylem is specified early as an axis of vessel element cell files, whereas phloem is established through a set of asymmetric cell divisions also contributing to the intervening procambial tissue. **Yrjö Helariutta** (Sainsbury Laboratory, Cambridge University) showed that auxin and cytokinins interact to specify the xylem and phloem/procambia domains, respectively. Auxin promotes expression of the class III HD-ZIP genes to promote xylem identity. By taking advantage of a callose synthase-based genetic tool to control plasmodesmatal trafficking, Yrjö Helariutta's lab identified a group of mobile transcription factors that mediate the specification of pholem/procambial domain downstream of cytokinin. **Ari Pekka Mähönen** (University of Helsinki) showed that phloem and xylem originate from the proliferate activity of vascular cambium in a spatiotemporal pattern. Using a two-step CRE-lox-based clonal activation system, he showed that physical contact with existing xylem is important for cambium formation and that xylem pole pericycle (XPP) cell lineage can produce all the tissue types developed during secondary growth. Furthermore, he also found that cytokinin (CK) is essential for cambium activation (XPP and procambium).

REGENERATION

Plants are sessile organisms that cannot escape from damage. Plants have evolved amazing regenerate ability to survive in ever-changing environments. Classic work has shown that the balance between auxin and cytokinin controls callus formation and fate, i.e., whether shoot or root would grow out of callus. A recent breakthrough indicates that callus is derived from pericycle or related cells in the vascular tissue, and that the lateral root formation pathway is used for callus formation during regeneration.

At this conference, our knowledge of the molecular mechanisms underlying both root and shoot regeneration has been moved forward. **Lin Xu** (Shanghai Institute of Plant Physiology & Ecology) suggested that two steps are required to establish an adventitious root primordium during *de novo* root organogenesis from leaf explants. The first step involves auxin-induced expression of *WOX11* and *12* to convert regeneration-competent cells into root founder cells. Then, WOX11 and 12 directly activate the expression of *WOX5*, *WOX7*, and *LBD16* to initiate root primordia. It has long been known that cytokinin promotes shoot formation during regeneration. **Xian Sheng Zhang** (Shandong Agricultural University) showed that B-type ARRs, a key cytokinin signaling pathway component, directly bind to *WUS* promoter to *de novo* activate its expression and trigger shoot meristem induction. Thus, the same molecular mechanism activating *WUS* expression *de novo* is utilized both in regeneration and in AM and FM initiation. Furthermore, B-type ARRs suppress the expression of *YUC* genes, encoding auxin biosynthetic enzymes. The suppression of *YUC*s may result in a local low auxin environment critical for shoot meristem initiation. The regenerative capacity declines with age. **Jia-Wei Wang** (Shanghai Institute of Plant Physiology & Ecology) showed that an age-regulated microRNA, miR156, regulates shoot regenerative capacity through modulating cytokinin signaling. In old plants, the miR156-targeted SPL transcription factor heterodimerizes with B-type ARRs to attenuate cytokinin signaling, thus reducing shoot regenerative capacity. To further dissect the regeneration pathway, **Jian Xu** (National University of Singapore) developed an enhancer-trap population to screen for lines that do not require the use of hormones to convert lateral root primordia into shoot meristems.

After an open discussion, all agreed to turn this conference into a meeting series for the meristem biology community. The 2nd International Conference on Plant Meristem Biology will be held in September, 2018, located in Hefei, China. Zhong Zhao will be in charge of the local organization for the conference. Furthermore, the 3rd Meristem Biology meeting has been tentatively scheduled in Cambridge, UK, in 2020.

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