# Schedule for "2024 International Symposium on Plant

# **Environmental Resilience** "

| Aug 06-07, 2024                   |   |  |  |
|-----------------------------------|---|--|--|
| Lecture Hall, College             | e of Biological Sciences, China Agricultural University,  |  |  |
| Beijing, China                    |   |  |  |
| Aug 06, Tuesday, 20               | Aug 06, Tuesday, 2024   |  |  |
| 9:00-9:10                         | <b>Opening Ceremony</b><br>Welcome address by <b>Shuhua Yang</b><br>(China Agricultural University, China)  |  |  |
| <b>9:10-10:10</b><br>9:10-9:45    | <u>Session I (Chair: Guozhi Bi)</u><br>Dae-Jin Yun (Konkuk University, South Korea)<br>Cold stress induced dynamic chromatin accessibility<br>in <i>Arabidopsis</i>                                     |  |  |
| 9:45-10:10                        | <b>Yongliang Zhang</b> (China Agricultural University,<br>China)<br>Proxitome profiling reveals novel components of<br>antiviral innate immune signaling pathways in<br>plants                          |  |  |
| 10:10-10:40                       | Group photograph/Coffee and Tea Break   |  |  |
| <b>10:40-11:55</b><br>10:40-11:15 | <b>Session II</b> (Chair: <b>Caifu Jiang</b> )<br><b>Woe-Yeon Kim</b> (Gyeongsang National University,<br>South Korea)<br>Diurnal regulation of root growth by circadian clock<br>in <i>Arabidopsis</i> |  |  |
| 11:15-11:40                       | <b>Liang Ma</b> (China Agricultural University, China)<br>Rhizosphere microorganisms enhance RLCK1-<br>mediated SOS2 phosphorylation and activation to<br>confer salt tolerance in plants               |  |  |

| 11:40-11:55                       | <b>Beibei Liu</b> (NanoTemper Technology (Beijing) Co.,<br>LTD)   |
|-----------------------------------|---|
|                                   | Monolith spectral shift technology empowers the study of plant environmental resilience   |
| 11:55-14:00                       | Lunch Break   |
| 14:00-14:30                       | Poster Session  |
| <b>14:30-15:30</b><br>14:30-15:05 | <u>Session III (Chair: Yiting Shi)</u><br>Isabel Baürle (University of Potsdam, Germany)<br>How plants remember a stressful day – a role for<br>chromatin-based mechanisms            |
| 15:05-15:30                       | <b>Yanglin Ding</b> (China Agricultural University, China)<br>Mechanism of heat stress responses regulated by<br>receptor-like kinases in plants                                      |
| 15:30-15:50                       | Coffee and Tea Break  |
| <b>15:50-17:40</b><br>15:50-16:25 | <u>Session VI (Chair: Jing Zhang)</u><br>Kyuha Choi (Pohang University of Science and<br>Technology, South Korea)<br>Control of meiotic crossover patterning in<br><i>Arabidopsis</i> |
| 16:25-17:00                       | <b>Michael Lenhard</b> (University of Potsdam, Germany)<br>Telling left from right - the genetic and molecular<br>basis of enantiostyly in <i>Wachendorfia</i> and <i>Barberetta</i>  |
| 17:00-17:40                       | Short talks by postdocs and students ( $5 \min 	imes 8$ )   |

### Aug 07, Wednesday, 2024

| <b>9:00-10:25</b><br>9:00-9:35    | <u>Session V (Chair: Jigang Li)</u><br>Young Hun Song (Seoul National University, South<br>Korea)<br>Effect of red to far-red light on development and<br>stress responses in <i>Arabidopsis</i>  |
|-----------------------------------|---|
| 9:35-10:00                        | <b>Jigang Li</b> (China Agricultural University, China)<br>Phosphorylation mechanism of the phyA<br>photoreceptor   |
| 10:00-10:25                       | <b>Wen Song</b> (China Agricultural University, China)<br>Biochemical mechanism of TIR-triggered plant<br>immunity  |
| 10:25-10:45                       | Coffee and Tea Break  |
| <b>10:45-11:50</b><br>10:45-11:10 | <u>Session VI (Chair: Wenkun Zhou)</u><br>Wenkun Zhou (China Agricultural University,<br>China)<br>Salt signaling directly regulates PLETHORA to<br>maintain root meristem  |
| 11:10-11:50                       | Short talks by postdocs and students ( $5 \min 	imes 8$ )   |
| 11:50-14:00                       | Lunch Break   |
| <b>14:00-15:35</b><br>14:00-14:35 | <u>Session VII (Chair: Ling Xu)</u><br>Jeremy Murray (Center for Excellence in Molecular<br>Plant Sciences, Chinese Academy of Sciences, China;<br>John Innes Centre, UK)<br>Sending the right signals: Flavonoid specificity in<br>the legume-rhizobia symbiosis |
| 14:35-15:00                       | <b>Pengbo Liang</b> (China Agricultural University,<br>China)<br>Evolutionarily conserved Formin proteins facilitate<br>intracellular symbiotic microbe infections in legume<br>and non-legume plants   |
| 15:00-15:35                       | <b>Takuya Suzaki</b> (University of Tsukuba, Japan)<br>How plants obtain nitrogen by supplying iron to<br>symbiotic bacteria  |

| 15:35-15:55 | Coffee and Tea Break  |
|-------------|---|
| 15:55-17:30 | Session VIII (Chair: Liangsheng Wang)   |
| 15:55-16:30 | <b>Dario Leister</b> (University of Munich, Germany)<br>Photosynthetic cyclic electron flow   |
| 16:30-16:55 | <b>Liangsheng Wang</b> (China Agricultural University,<br>China)<br>Chloroplast singlet oxygen-induced retrograde<br>signaling relies on TOC33-mediated EX1-UVR<br>transportation in <i>Arabidopsis</i> |
| 16:55-17:30 | Award Ceremony (Chair: Yi Wang)   |
| 17:30-17:40 | <b>Closing Remarks</b> by <b>Yan Guo</b><br>(China Agricultural University, China)  |

#### Cold stress induced dynamic chromatin accessibility in Arabidopsis

#### Dae-Jin Yun\*

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#### Abstract



Plants exposed to various environmental stresses, including cold temperature, undergo differential gene expression, and activate stress response pathways by various molecular mechanisms including chromatin remodeling. Here, we used genome wide chromatin accessibility studies to investigate the dynamic changes on chromosomes in *Arabidopsis*. We show the heterochromatin structure was lost in the nucleus upon cold exposure. There was gain in chromatin accessibility throughout the genome and that this was associated with histone reorganization in response to cold stress. In addition, we observed differential gene expression that did not correspond with gain in chromatin accessibility upon cold stress. The differential gene expression was mediated by positional CBFs/DREB1 (C-repeat Binding Factors/Dehydration-Responsive Element-Binding) transcription factor recruitment. Binding of CBFs to H2A.X.5 promoter led to its upregulation upon cold exposure. In contrast, binding of CBFs to H2A.W.12 enhancer led to its downregulation. Therefore, in response to increased chromatin accessibility under cold stress, transcription factor CBFs plays a critical role in regulating gene expression depending on its binding locations in the *Arabidopsis* genome.

# Proxitome profiling reveals novel components of antiviral innate immune signaling pathways in plants

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#### Abstract

The two most important layers of defense in plant antiviral immunity are RNAi and NLRmediated antiviral immunity. To uncover new components in these two defense systems, we used proximity labeling (PL) technology with the barley stripe mosaic virus silencing suppressor  $\gamma$ b and the tobacco mosaic virus resistance protein N NLR immune receptor as baits for quantitative proteomics analysis. We identified novel components of the RNAi and NLRmediated antiviral immune signaling pathways and elucidated their molecular mechanisms. Specifically:

- 1. RNA silencing plays a crucial role in defending against viral infections in diverse eukaryotic hosts. Despite extensive studies on core components of the antiviral RNAi pathway such as DCLs, AGOs, and RDRs proteins, host factors involved in antiviral RNAi remain incompletely understood. Using the barley stripe mosaic virus (BSMV)-encoded γb, a viral suppressor of RNA silencing (VSR), as the bait protein, we identified the DEAD-box RNA helicase RH20, a broadly conserved protein in plants and animals with a homologous human protein known as DDX5. We found that RH20 positively regulates RNAi-based antiviral immunity in plants by associating with SGS3/RDR6 bodies, while BSMV γb VSR subverts the RH20-mediated antiviral defense by interfering with the RH20-SGS3 interaction.
- SGT1 is highly conserved across various eukaryotes and associates with the SKP1-Cullin-F-box (SCF) E3 ubiquitin ligase complex. However, its molecular regulatory network and whether it targets immune repressors to coordinate plant immune activation remain undefined. Using proximity labeling (PL) techniques, we constructed the SGT1 interaction network and identified a conserved SGT1-NSL1 signaling module that activates NLRmediated immunity.

In summary, our results uncover novel components in antiviral innate immune signaling pathways and provide new potential targets for controlling plant viral diseases.

# Diurnal regulation of root growth by circadian clock in *Arabidopsis* <u>Woe-Yeon Kim</u>

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#### Abstract

The circadian clock is an internal timekeeper that ensures efficient energy utilization in most living organisms through periodicity. In plants, biological clocks orchestrate the timing of energy use to optimize growth. The evolutionarily conserved energy sensor, TARGET OF RAPAMYCIN (TOR), activates glucose-mediated plant growth and development by stimulating meristem activation. This raises the possibility that glucose-TOR signaling cycles between activation and inhibition modes govern the diurnal dynamics of plant growth, though this is still largely unknown. Using mathematical modeling along with genetic and biochemical approaches, we discovered that the circadian clock component GIGANTEA (GI) negatively regulates glucose-TOR signaling that mediates meristem activation. Interestingly, GI cooperates with FK506-BINDING PROTEIN 12 (FKBP12) in the inhibition mode of glucose-TOR signaling and mediates the interaction between the FKBP12-rapamycin binding (FRB) domain of TOR and FKBP12 without rapamycin in planta. Remarkably, we found that TOR phosphorylates GI at serine 911 (S911), and this phosphorylation is essential for GI to bind to the kinase domain of TOR and inhibit root growth. This suggests that TOR-mediated GI phosphorylation acts as negative feedback for root meristem activation. Our findings demonstrate that GI regulates glucose-TOR signaling to mediate diurnal meristem activation through negative feedback regulation. Collectively, the TOR-GI-FKBP12 axis controls the diurnal dynamics of plant growth.

# Rhizosphere microorganisms enhance RLCK1-mediated SOS2 phosphorylation and activation to confer salt tolerance in plants <u>Liang Ma</u>\*

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#### Abstract

The evolutionarily conserved SALT OVERLY SENSITIVE (SOS) pathway is essential for plant adaptation to salt stress. In this study, we show that SOS2, the key kinase of the SOS signaling pathway is phosphorylated by a Receptor-like Cytoplasmic Kinase 1 (RLCK1). RLCK1-mediated phosphorylation of SOS2 is essential for relieving SOS2 auto-inhibition, thus promoting SOS2 activity and conferring the enhanced salt tolerance. Loss of RLCK1 renders plants salt sensitive and the activation of RLCK1-SOS2 module is regulated by rhizosphere microorganisms or salt-induced plant elicitor peptides, which is independent of the calcium sensors SOS3 and SCaBP8. Together, our study demonstrates that rhizosphere microorganisms- or small peptides-activated RLCK1 promotes plant salt tolerance by increasing SOS2 activity, thus broadening our understanding of how plants adapt to salt stress according to their diverse soil microenvironment; and providing the insight into signaling plasticity underlying biotic or abiotic stress cross-tolerance in plants.

# How plants remember a stressful day-a role for chromatin-based mechanisms

#### Isabel Bäurle\*

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#### Abstract

In nature, plants often encounter chronic or recurring stressful conditions. An increasing number of observations suggest that plants can be primed by exposure to stress, thereby activating a stress memory that enables a more efficient response upon a recurring stress incident. My lab studies heat stress memory in plants as a model case for environmental stress memory. Seedlings acquire thermotolerance through a heat treatment at sublethal temperatures (priming heat stress) that enables them to survive an otherwise lethal heat stress. This thermotolerance is actively maintained for several days as indicated by the existence of mutants which are able to establish thermotolerance, but fail to maintain it. Heat stress induces sustained histone methylation at heat stress memory-related loci, marking them as recently transcriptionally active. This methylation requires the activity of specific heat-shock transcription factors. In addition, it requires the kinase module of the Mediator co-activator complex to mediate enhanced transcriptional activation after reinduction. Hence, complex interactions between different levels of transcriptional and epigenetic regulation sustain transcriptional memory.

# Mechanism of heat stress responses regulated by receptor-like kinases in plants

#### Jing Li, Shuhua Yang, Yanglin Ding\*

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#### Abstract

With global warming, heat stress causes adverse effects on plant growth and productivity, which is becoming an increasingly significant problem for food security. Previous studies have elucidated the transcriptional regulatory networks involved in heat stress. However, it remains unclear how plant responds to early heat stress signal.

Here, we report that two receptor-like kinases including HRK1 and HRK2 form a complex to regulate heat-induced ROS bursts and thus enhances thermotolerance in plants. HRK1 is a receptor-like cytoplasmic protein kinase that is rapidly activated upon heat stress within 5 minutes and then phosphorylates the N terminus of RBOHD and stabilizes RBOHD during the heat, thereby resulting in ROS bursts and subsequent heat signal activation. Moreover, the LRR-RLK HRK2 is also rapidly activated by the heat dependently on small peptide HRP1, which is critical for HRK1 activation under heat stress. Mutation of both *HRK1* and *HRK2* causes significant ROS reduction and impaired thermotolerance in plants. This study thus elucidates central mechanism by which the peptide–RLK complex activates heat stress response and resistance.

#### Control of meiotic crossover patterning in Arabidopsis

#### Heejin Kim, Jaeil Kim, Namil Son, Kyuha Choi\*

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#### Abstract



Meiosis is fundamental to eukaryotic reproduction. During meiosis homologous chromosomes pair up and undergo reciprocal exchanges, called crossovers. Meiotic crossover has a significant impact on genetic diversity and breeding in plants. Crossovers are tightly regulated, with one obligate exchange forming per chromosome pair, and multiple crossovers are rarely formed and widely spaced by crossover interference. In Arabidopsis thaliana, crossover number and distribution can be explained by a diffusion-mediated coarsening model. In this model, large, approximately evenly-spaced foci of the pro-crossover ubiquitin E3 ligase HEI10 grow at the expense of smaller, closely-spaced clusters and eventually concentrate at designated crossover sites. However, the molecular mechanisms that control the abundance and dynamics of HEI10 remain unexplored. We performed a forward genetic screen in Arabidopsis to identity new regulators of crossover patterning using fluorescence-tagged crossover reporters. We isolated and characterized three high crossover rate (hcr) mutants, hcr1, hcr2, and hcr3, that increase meiotic crossover genome-wide and decrease interference strength. Using a comprehensive array of cytogenetics, genetics, genomics, biochemical, and mathematical modeling approaches, we demonstrated that HCR1/PPX1 phosphatase and HCR3/J3 co-chaperone facilitate the proteolysis of HEI10, while HCR2/HSBP, a negative regulator of HSFs, represses the transcription of HEI10. Our findings provide new insights into how HCR1/2/3 anti-crossover factors mediate crossover interference and limit crossovers via transcriptional and posttranslational control of HEI10. In addition, our works sheds light on the potential of controlling HCR1/2/3 to unleash genetic diversity for plant breeding.

# Telling left from right - the genetic and molecular basis of enantiostyly in Wachendorfia and Barberetta

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#### Abstract

Telling left from right is a difficult task for organisms, as the two

sides can only defined by reference to two other axes. While genetically encoded left-right asymmetry is found in many animals, it is very rare in plants. One example is the mirror-image flowers of Wachendorfia and Barberetta. Species of these two closely related genera show enantiostyly, i.e. they form flowers with the style deflected to the left of the midline and one of the three stamens to the right, or with the opposite arrangement. All flowers on one individual have the same handedness, indicating genetic control. Functionally, the reciprocal placement of the stigma and the opposing anther promotes pollen placement on segregated sites of the pollinators' bodies and efficient transfer to stigmas of the opposite morph. We are investigating the genetic, molecular and developmental basis of mirror-image style and stamen deflection in Wachendorfia and Barberetta. Genome assemblies and pooled sequencing of left- and rightmorph individuals from natural populations have identified a hemizygous region whose presence causes right-styled flowers, whereas plants lacking this region form left-styled flowers. This region contains two conserved genes throughout all the species tested, one of which is expressed in the developing stamen, the other in the style around the time when the organs begin to deflect from the midline. Efforts to assign functions to these genes are ongoing. Identification of the hemizygous region opens up the possibility of assaying realized mating patterns in natural populations and testing the efficiency of enantiostyly in promoting disassortative mating. Thus, our results pave the way for a mechanistic understanding of leftright asymmetry in plants and its impact on plant reproduction.

# Effect of red to far-red light on development and stress responses in *Arabidopsis*

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#### Abstract

Plants have evolved the ability to adjust their growth and development in response to the challenges posed by a wide range of fluctuating environments. Various physiological adaptations are closely related to changes in red (R) or far-red (FR) light and R:FR ratios. For example, flowering in Arabidopsis is inhibited under red light-enriched growth conditions but promoted under far-red light-enriched conditions. Therefore, a plant's ability to respond to or resist environmental stress is crucial in tailoring its growth and development to specific ecological conditions. However, this adaptability often comes at the cost of increased sensitivity to other forms of stress. For instance, the shade avoidance response enhances a plant's capacity to adapt to low R:FR light conditions but concurrently diminishes its immune defenses against pathogens, ultimately impacting productivity. Given that these stresses commonly coexist in agricultural settings, fortifying resistance to multifaceted stressors becomes paramount for enhancing overall productivity. In this seminar, I will introduce recent research findings on the molecular basis of flowering time regulation and shade avoidance response in Arabidopsis, where far-red light acts as a key inducer. By elucidating the mechanisms of time-specific expression regulation of the FLOERING LOCUS T gene by far-red light and the regulation mechanism of leaf hyponasty mediated by far-red light, we aim to provide insights that can contribute to the development of crops with enhanced resilience to abiotic stress environments.

#### Phosphorylation mechanism of the phyA photoreceptor

Ziyi Feng<sup>1,7</sup>, Meijiao Wang<sup>1,7</sup>, Yan Liu<sup>1</sup>, Cong Li<sup>1</sup>, Shaoman Zhang<sup>1</sup>, Jie Duan<sup>1</sup>, Jiaqi Chen<sup>1</sup>, Lijuan Qi<sup>1,2</sup>, Yanru Liu<sup>1</sup>, Hong Li<sup>1</sup>, Jie Wu<sup>3</sup>, Yannan Liu<sup>4</sup>, William Terzaghi<sup>5</sup>, Feng Tian<sup>6</sup>, Bojian Zhong<sup>4</sup>, Xiaofeng Fang<sup>3</sup>, Weiqiang Qian<sup>2</sup>, Yan Guo<sup>1</sup>, Xing Wang Deng<sup>2</sup>, and **Jigang Li<sup>1,\*</sup>** 

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These authors contributed equally: Ziyi Feng, Meijiao Wang.



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#### Abstract

Phytochrome A (phyA) is the plant far-red (FR) light photoreceptor that plays an essential role in regulating photomorphogenic development in FR-rich conditions, such as canopy shade. It has long been observed that phyA is a phosphoprotein *in vivo*, however, the protein kinases that could phosphorylate phyA remain largely unknown. Here, we show that a small protein kinase family, consisting of four members named PHOTOREGULATORY PROTEIN KINASES (PPKs; also known as MUT9-LIKE KINASES [MLKs]), directly phosphorylate phyA in vitro and in vivo. In addition, TANDEM ZINC-FINGER/PLUS3 (TZP), a recently characterized phyA-interacting protein required for in vivo phosphorylation of phyA, is also directly phosphorylated by PPKs. We uncover that TZP contains two intrinsically disordered regions in its N-terminal domain that undergo liquid-liquid phase separation (LLPS) upon light exposure. Notably, the LLPS of TZP promotes colocalization and interaction between PPKs and phyA, thus facilitating PPK-mediated phosphorylation of phyA in FR light. Together, our study identifies PPKs as the first class of protein kinases mediating phosphorylation of phytochrome A and demonstrates that the LLPS of TZP contributes significantly to more production of the phosphorylated phyA form in FR light.

#### **Biochmical mechanism of TIR-triggered plant immunity**

#### Wen Song\*

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# Abstract



Toll-interleukin-1 receptor (TIR) domain is a conserved immune module in animals, plants and bacteria. Plant nucleotide-binding leucine-rich repeat (NLR) immune receptors with an N-terminal TIR domain recognize pathogen effectors to trigger host cell death, known as the hypersensitive response (HR), to limit pathogen spread. However, the biochemical mechanism by which TIR-NLR triggers cell death is unclear. Here we show that pathogen effector-activated TIR-NLRs form oligomers (resistosomes) to enable TIR enzymatic activity. TIR domains catalyze NAD<sup>+</sup>/ATP to generate immune small molecules pRib-AMP/ADP and di-ADPR/ADPr-ATP. The TIR-catalyzed small molecules specifically bind to EDS1 family proteins to stimulate their interaction with coiled-coil NLR (CC-NLR) ADR1 and NRG1, leading to Ca<sup>2+</sup> influx and cell death. We also uncovered a novel activation mechanism for the noncanonical NLR TIR domain proteins that lack effector binding domains. Substrate NAD+/ATP binding induces TIR domain proteins forming condensates, thereby assembling TIR oligomers identical to TIR-NLR resistosomes. In addition, we unexpectedly found that TIR domain can hydrolyze RNA/DNA to produce 2'3'cNMP to promote immunity. Taken together, we revealed the biochemical mechanism of TIR-conferred immunity in plants.

# Salt signaling directly regulates PLETHORA to maintain root meristem

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#### Abstract

Plants respond to a changing environment by reallocating their investments into growth and response to external stresses. As a consequence, plants display a considerable amount of developmental plasticity. Mounting evidence suggests that this trade-off is regulated by the convergence of developmental and environmental signalling networks in specific regions of plant growth. The continuous production of cells in these regions is fuelled by stem cells in the root and shoot apical meristem. We have gained an increasing knowledge about environmental control of overall plant growth, and our previous studies have shown that stress hormones regulate the division rate of organizer cells in the root stem cell niche, and also regulate root tip regeneration. But little is known about how root stem cells maintain root growth to counteract abiotic stresses.

Salt stress is one of the unfavorable environmental factors to affect plants. Salinity represses root growth, resulting in reduced biomass of agricultural plants. The AP2-domain transcription factors PLETHORA1/2 (PLT1/2) act as master regulators in root meristem maintenance in *Arabidopsis*. We report that the salt overly sensitive (SOS) pathway component SOS2 regulates PLT1/2 at the post transcriptional level, critical for root apical meristem maintenance under salt stress. Moreover, SOS2-mediated PLT1/2 phosphorylation improves root growth recovery after salt stress alleviation. Our research highlights a SOS2-PLT1/2 core protein module that is required for protecting primary root growth and meristem maintenance from salt stress.

## Sending the right signals: Flavonoid specificity in the Legume-Rhizobia symbiosis

Yiting Ruan<sup>1,2</sup>, Shangzhi Dong<sup>1</sup>, Suyu Jiang<sup>1,2</sup>, Yisheng Wang<sup>1,2</sup>, Yuxin Zhuang<sup>1,2</sup>, Xiaotian Wu<sup>1,2</sup>, Yu Zhang<sup>1</sup>, **Jeremy D. Murray**<sup>1,2\*</sup>

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#### Abstract

The symbiosis of legumes with nitrogen-fixing bacteria is often highly specific, with many legumes only forming nodules with a limited subset of rhizobia. In the *Medicago truncatula-Sinorhizobium* symbiosis, this specificity is determined by rhizobial signaling molecules called Nod factors and specific host flavonoids that induce their production through activation of NodD lysR transcription factors. Through structural and mutational analyses, we have dissected the basis for the recognition of specific flavonoids by NodD. This provides a basis for understanding the evolution of nodulation specificity in legumes and provides new tools that can be used to establish novel, highly specific plant-microbe interactions.

### Evolutionarily conserved Formin proteins facilitate intracellular symbiotic microbe infections in legume and non-legume plants

Lijin Qiao<sup>1,2†</sup>, Heng Sun<sup>1,2†</sup>, Casandra Hernández-Reyes<sup>3,4</sup>, Eija Schulze<sup>3,4</sup>, Xia Xu<sup>1</sup>, Yutian Jia<sup>1</sup>, Julian Knerr<sup>5</sup>, Tak Lee<sup>6,7</sup>, Jean Keller<sup>8</sup>, Cyril Libourel<sup>8</sup>, Robert Grosse<sup>5</sup>, Lin Zhang<sup>9</sup>, Guanghui Yang<sup>1</sup>, Changfu Tian<sup>1,2</sup>, Giles E.D. Oldroyd<sup>6,7</sup>, Pierre-Marc Delaux<sup>8</sup>, Thomas Ott<sup>3,4\*</sup>, **Pengbo Liang<sup>1,2\*</sup>** 

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<sup>7</sup>Crop Science Centre, Department of Plant Sciences, University of Cambridge, UK
<sup>8</sup>Laboratoire de Recherche en Sciences Végétales (LRSV), Université de Toulouse, CNRS, France
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<sup>†</sup> L.O. and H.S. contributed equally to this work

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#### Abstract

Plants deploy specific cell-surface-cytokinetic to counteract the invasion of intricate microbes. Like legume root hair infections own specific morphological inventions that entrap the symbiont, particularly an invaginated foci aided by cytoskeleton for rhizobia intracellular entry. How hosts manipulate biophysical forces that pull down the cell membrane to invaginate remains unknown. Our investigations uncovered an actin nucleating Formin protein, termed INFO, as indispensable for the intracellular infection by rhizobia in the model legume species Medicago truncatula. INFO condensates in the symbiotic-specific nanodomain at the plasma membrane, facilitating the actin assembly required for symbiont entry. Given that INFO is evolutionarily not exclusive to the nodulating lineage but well confined among Arbuscular Mycorrhiza-forming species, its parallel function in AMF intracellular infection is then substantiated in both Medicago and non-legume crop tomato Solanum lycopersicum. Additionally, the core root nodule symbiosis transcription factor NIN, however, can activate the tomato-derived INFO, evidencing evolutionary retention of rhizobia-responsive signature in non-legume. Our findings bolster understanding of the molecular dialogues underpinning symbiotic associations and propose a framework in which non-legume nitrogen fixation likely could be engineered by leveraging the common symbiosis signaling toolkit.

#### How plants obtain nitrogen by supplying iron to symbiotic bacteria

#### Takuya Suzaki<sup>1,2\*</sup>

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#### Abstract



Legumes can utilize nitrogen in the atmosphere by establishing a symbiotic relationship called root nodule symbiosis (RNS) with nitrogen-fixing rhizobia. While RNS is largely beneficial for plants, it involves energy-consuming processes. Thus, plants are capable of controlling RNS depending on nitrogen availability. Despite the recent understanding of the molecular basis of external nitrate-mediated control of RNS, it remains mostly elusive how plants regulate RNS depending on internal nitrogen status. In addition, iron has an essential role in symbiotic nitrogen fixation to function as a cofactor of leghemoglobin, an oxygen-carrying protein required for symbiotic nitrogen fixation, and nitrogenase that catalyzes nitrogen fixation; however, the mechanism of iron accumulation in nodules is poorly understood.

Here, we focus on the transcriptome in response to internal nitrogen status during RNS in *Lotus japonicus* and identify that IRON MAN (IMA) peptide genes are expressed depending on symbiotic nitrogen fixation. We show that *LjIMA1* and *LjIMA2* expressed in the shoot and root play systemic and local roles in concentrating internal iron to the nodule. Our data indicate that they act according to plant iron demand and have an essential role in iron accumulation in nodules to establish RNS. We also show that *L japonicus* NODULE INCEPTION (LjNIN), a master transcription factor of nodulation, regulates *LjIMA1* expression, indicating a direct molecular link between the iron acquisition mechanism and an important nodulation signaling pathway. Furthermore, IMA peptides have conserved roles in regulating nitrogen homeostasis by adjusting nitrogen-iron balance in *L. japonicus* and *Arabidopsis thaliana*. These findings indicate that IMA-mediated iron provision plays an essential role in regulating nitrogen-related physiological processes.

#### Photosynthetic cyclic electron flow

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#### Abstract

Cyclic electron flow has been known for over 60 years, but its components, mechanism, and regulation are still largely elusive. PROTON GRADIENT REGULATION5 (PGR5) is believed to promote cyclic electron flow. Its deficiency impairs photosynthetic control and increases photosensitivity of photosystem (PS) I, leading to lethality under fluctuating light. Additionally, two proteins, PGRL1 and PGRL2, regulate the activity of PGR5. PGRL1 channels the activity of PGR5, while PGRL2 causes the removal of PGR5 if PGRL1 is absent. This leads to the peculiar situation where PGR5 does not accumulate without PGRL1, but in the absence of both PGRL1 and PGRL2, PGR5 can accumulate and promote cyclic electron flow. To gain a better understanding of the role of PGR5 and PGRL1, we conducted a screen for suppressor mutations that rescue the lethality of *pgr5* or *pgr11* plants under fluctuating light. A portfolio of mutations in various genes has been identified to affect PSII function, cytochrome  $b_6f$  assembly, plastocyanin accumulation, the chloroplast FBPase, or one of its regulators. At least two instances were found in which the *pgr5* (or *pgr11*) mutation suppressed the effects of other mutations, revealing unexpected functional relationships. The results from the screen will be presented and discussed in this meeting.

## Chloroplast singlet oxygen-induced retrograde signaling relies on TOC33-mediated EX1-UVR transportation in *Arabidopsis*

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#### Abstract

Plant chloroplasts performing oxygenic photosynthesis inevitably generate certain amount of reactive oxygen species (ROS), especially under stress conditions. Among these ROS,  ${}^{1}O_{2}$  is the major one and most detrimental to the chloroplast. Chloroplast protein EX1 is a sensor of <sup>1</sup>O<sub>2</sub>, perceiving elevated <sup>1</sup>O<sub>2</sub> levels via its Trp643 oxidation. However, the following events after Trp643 oxidation and downstream components that relay  ${}^{1}O_{2}$  signal are still unknown. In the present study, we report isolation of SOF1 (suppressor of flu 1) in a screen for suppressor mutants using ethyl methanesulfonate (EMS) mutagenized flu (gEX1-Flag/flu) seeds. In *flu/sof1*, all the <sup>1</sup>O<sub>2</sub>-induced stress responses are largely supressed, but the amount of  $^{1}O_{2}$ generated is similar to that in the *flu* mutant. SOF1 encodes the chloroplast outer envelope anchored preprotein import receptor TOC33. Mutation of TOC33 neither impairs the import and location of EX1 protein nor affects <sup>1</sup>O<sub>2</sub>-induced proteolysis of EX1 protein in the chloroplast, but blocks <sup>1</sup>O<sub>2</sub>-induced chloroplast-to-nucleus retrograde signalling. Meanwhile, we found that the UVR domain of EX1 protein interacts not only with TOC33 in the chloroplast, but also interacts with WRKY transcription factors in the nucleus, activating expression of singlet oxygen responsive genes (SORGs). Our current study demonstrates that SOF1/TOC33 protein positively mediates chloroplast <sup>1</sup>O<sub>2</sub>-EX1 signalling, and that ectopic expression of EX1-UVR domain in the nucleus is sufficient to activate expression of SORGs and the following stress responses.

# NOTES: