# 2023植物逆境应答与环境适应性学术研讨会

# 2023 Symposium on Plant Responses to Abiotic Stresses and Environmental Signals

Aug 14 (Mon) - Aug 17 (Thu), 2023
China Agricultural University
Beijing, China

Organizer: State Key Laboratory of Plant Environmental Resilience,
College of Biological Sciences, China Agricultural University
Funding: 111 Project, Ministry of Education of the People's Republic of China

# Schedule for Workshop on "Plant Responses to Abiotic Stresses and Environmental Signals"

***Aug 15-16, 2023***

***Lecture Hall, College of Biological Sciences, China Agricultural University, Beijing***

***Aug 15, Tuesday, 2023***

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| **8:30-8:40** | **Opening Ceremony**<br>Welcome address by **Shuhua Yang** (China Agricultural University, China) |
| **8:40-9:50** | **Session I** (Chair: **Caifu Jiang**) |
| 8:40-9:15 | **Jörg Kudla** (Universität Münster, Germany)<br>A bi-kinase module sensitizes and potentiates NOX activation in systemic immune signaling |
| 9:15-9:50 | **Dae-Jin Yun** (Konkuk University, South Korea)<br>The HOS15-HDA9 complex associates with HYL1 to modulate miRNA expression in response to ABA signaling |
| 9:50-10:10 | *Coffee and Tea Break* |
| **10:10-11:50** | **Session II** (Chair: **Jigang Li**) |
| 10:10-10:45 | **Richard Vierstra** (Washington University in St. Louis, USA)<br>Phytochrome photoactivation and signaling: finally light at the end of the tunnel |
| 10:45-11:20 | **Nam-Chon Paek** (Seoul National University, South Korea)<br>Inactivation of rice *LOV KELCH REPEAT PROTEIN 2* enhances drought tolerance by increasing cuticular wax biosynthesis |
| 11:20-11:30 | 王培（北京新梁科技有限公司）<br>KASP基因分型系统和eBlot成像系统的应用 |

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| 11:30-11:40 | 纪玉锶（北京麦科伦科技有限公司）<br>基于DynaPlant动态成像分析系统 |
| 11:40-11:50 | 王楠楠（赛默飞世尔科技(中国)有限公司）<br>育种发展中多维度农业基因组学解决方案 |
| 11:50-14:00 | *Lunch Break* |
| **14:00-15:10** | **Session III** (Chair: **Wenkun Zhou**) |
| 14:00-14:35 | **Wenbo Ma** (The Sainsbury Laboratory, Norwich, UK)<br>Cell type-specific responses to fungal infection in plants revealed by single-cell transcriptomics |
| 14:35-15:10 | **Jian Xu** (Radboud University, The Netherlands)<br>From single cells to multicellular life and vice versa |
| 15:10-15:30 | *Coffee and Tea Break* |
| **15:30-17:15** | **Session VI** (Chair: **Liangsheng Wang**) |
| 15:30-16:05 | **Chanhong Kim** (Shanghai Center for Plant Stress Biology, China)<br>GENOMES UNCOUPLED1-dependent biogenic thermal stress responses |
| 16:05-16:40 | **Bernhard Grimm** (Humboldt-Universität zu Berlin, Germany)<br>Multiple posttranslational control mechanisms at regulatory focal points of tetrapyrrole biosynthesis |
| 16:40-17:15 | **Ralph Bock** (Max Planck Institute of Molecular Plant Physiology, Germany)<br>How the environment causes genetic changes in plants |

***Aug 16, Wednesday, 2023***

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| **9:00-10:10** | **Session V** (Chair: **Ling Xu**) |
| 9:00-9:35 | **Omri Finkel** (The Hebrew University of Jerusalem, Israel)<br>Establishing causality in the rhizosphere using microbiome deconstruction |

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| 9:35-10:10 | **Shaul Yalovsky** (Tel Aviv University, Israel)<br>Small G protein signaling: bridging the gap between pattern formation and abiotic stress |
| 10:10-10:30 | *Coffee and Tea Break* |
| **10:30-11:40** | **Session VI** (Chair: **Yiting Shi**) |
| 10:30-11:05 | **Byeong-Ha Lee** (Sogang University, South Korea)<br>Arabidopsis PRP6 homolog STA1-mediated gene expression is important for thermal stress tolerance |
| 11:05-11:40 | **Juan Dong** (Rutgers University, USA)<br>Precisions in time and space: stomatal production in Arabidopsis |
| 11:40-14:00 | *Lunch Break* |
| **14:00-15:10** | **Session VII** (Chair: **Jing Zhang**) |
| 14:00-14:35 | **Rodrigo A Gutiérrez** (Pontificia Universidad Católica de Chile, Chile)<br>Dissecting nitrate responses in time and space |
| 14:35-15:10 | **Kun-hsiang Liu** (Massachusetts General Hospital, USA)<br>Exploring novel regulators in nitrate signaling |
| 15:10-15:30 | *Coffee and Tea Break* |
| **15:30-16:40** | **Session VIII** (Chair: **Pengbo Liang**) |
| 15:30-16:05 | **Yansong Miao** (Nanyang Technological University, Singapore)<br>2D-phase separation for plant signaling |
| 16:05-16:40 | **Rubén Rellán-Álvarez** (North Carolina State University, USA)<br>North, south, up and down. The genes and molecules that allow maize to move all around |
| **16:40-16:50** | **Closing Remarks** by **Yan Guo** (China Agricultural University, China) |

# Abstracts

## How the environment causes genetic changes in plants

**Ralph Bock**

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**Abstract**

Changing environmental conditions are known to trigger alterations in gene expression programs to mediate adaptive responses. Whether stressful environmental conditions also cause genetic changes at the species and population levels is less well established. In my talk, I will present two examples of genetic processes that are sensitive to abiotic stress conditions and cause heritable changes in plants. In our research on experimental evolution, we reconstructed endosymbiotic gene transfer from the plastid (chloroplast) genome to the nuclear genome in the laboratory, and elucidated the underlying molecular mechanisms. We discovered that the escape of DNA from the chloroplast and its integration into the nuclear genome is sensitive to environmental stress. Specific abiotic stress conditions cause a strongly increased influx of organellar genes into the nuclear genome, demonstrating that challenging environmental conditions can accelerate genome evolution by stimulating intracellular DNA transfer. We further discovered that the inheritance of organelles and their genomes is sensitive to the environment. While chloroplast and mitochondrial genomes are normally maternally inherited in the vast majority of seed plants, specific abiotic stress conditions lead to a substantial level of biparental inheritance, thus providing opportunities for sexual recombination and competition between maternal and paternal organellar genomes in the progeny. The implications of these findings for genome evolution and population biology will be discussed.

**Key words:** abiotic stress, endosymbiotic gene transfer, maternal inheritance, organelle inheritance

# Precisions in time and space: stomatal production in Arabidopsis

**Juan Dong** [1,2]

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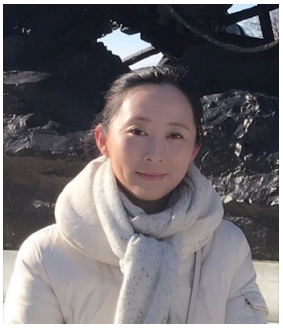

**Abstract**

Stomata are microscopic pores in the epidermis of the aerial organs of a plant. The mitogen-activated protein kinase (MAPK) cascades are critical signaling pathways that regulate diverse cellular processes by transmitting extracellular stimuli to intracellular machinery. Stomatal development and patterning in Arabidopsis are also controlled by a canonical MAPK cascade. The EPF peptide ligands are perceived by the cell surface leucine-rich repeat receptor-like proteins (LRR-RLPs) and kinases (RLKs). Activated RLP/RLK signaling triggers the downstream YDA-MKK4/5-MPK3/6 signaling cascade, which then regulates the substrate levels or activities, such as the bHLH SPEECHLESS transcription factor. Recent progress in our lab revealed new regulators and mechanisms that modulate the production of stomatal guard cells in Arabidopsis. My talk will discuss how these molecules feed into the regulation of components in the MAPK signaling-mediated signaling pathway to alter stomatal production in a spatiotemporal manner at the subcellular level.

**Key words:** stomatal development, MAPK signaling, subcellular localization, Arabidopsis

# Establishing causality in the rhizosphere using microbiome deconstruction

**Omri M Finkel**[1]

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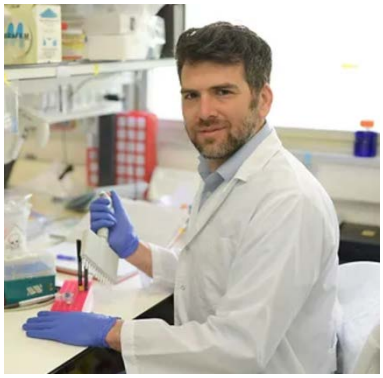

**Abstract**

Plants rely on sophisticated defense and immunity mechanisms to protect themselves from pests and pathogens. It is now established that the first layer of defense is provided by other organisms – the plant microbiota. Accumulating data suggest that the protection provided by the microbiota constitutes not just the plant's first line of defense, but possibly its most one, as disruptions to the microbiota render the plants susceptible to otherwise asymptomatic infections.

To understand how this layer of defense is deployed, we have been applying a realistically complex and fully tractable plant-soil-microbiome microcosm. This system provides a platform for the discovery of novel plant-beneficial traits, which only emerge within a microbial community context. In order to identify which components of the plant microbiota are critical for plant defense, we deconstructed this microcosm top-down, removing different microbial groups from the community to examine their effect on the plant. Applying this method reveals a high redundancy in the microbial protection of plants. Taxonomically disparate microbial consortia have similarly beneficial effects. Further dissection of the microbiota however, started to reveal unique roles for different microbe. We discovered the protective role of the genus *Variovorax*, which removed excess microbial auxins from the rhizosphere, and more recently, reinforced the crucial role the genus *Bacillus* plays in inhibiting pathogen growth in the plant.

We conclude that microbial protection of plants is layered, and that microcosm deconstruction is a powerful tool in unraveling its complexities.

**Key words**: plant immunity, microbiota, top-down, *Variovorax*, auxins

# Multiple posttranslational control mechanisms at regulatory focal points of tetrapyrrole biosynthesis

**Bernhard Grimm** and the Plant Physiology group at the Humboldt University

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**Abstract**

Tetrapyrrole biosynthesis (TPB) in plants consists of more than twenty enzymatic steps and is tightly controlled because of the synthesis of photoreactive intermediates and the different spatio-temporal demands of their end-products chlorophyll and heme. At hot spots of TPB many complementary posttranslational control mechanisms act on certain enzymes: The two enzymes glutamyl-tRNA reductase (GluTR) and glutamate-1-semialdehyde aminotransferase (GSAT) ensure the rate-limiting step of 5-aminolevulinic acid (ALA) synthesis, at the beginning of the TPB pathway. At the branch point to chlorophyll and heme synthesis, the Mg chelatase, a protein complex consisting of three different subunits, is responsible for forwarding protoporphyrin to chlorophyll synthesis. The light-dependent protochlorophyllide oxidoreductase requires also multiple control mechanisms to prevent substrate accumulation in darkness and organize oligomerisation for activity and stability during adverse environmental conditions. Thus, multiple factors for these posttranslational control mechanisms ensure the finetuned metabolic flow in TPB at the level of activity, stability, oligomerisation and subplastidal compartmentation of the contributing enzymes. In recent years, we successfully contributed to the elucidation of several complex control processes at these focal points during daytime, nighttime, and changing conditions in nature. The presentation summarizes some of these different regulatory strategies to control posttranslationally TBP by regulatory and supporting factors.

**Key words:** tetrapyrrole biosynthesis, chloroplast biogenesis, photosynthesis, posttranslational modification, 5-aminolevulinic acid, heme, plastid development *Arabidopsis*

# Dissecting nitrate responses in time and space

**Rodrigo A. Gutiérrez**

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**Abstract**

Nitrate is a nutrient and a potent signal that impacts global gene expression in plants. Regulatory factors controlling spatiotemporal nitrate responses are still largely unknown. In order to address this problem we assayed nitrate-responsive transcriptome changes in five major root cell types of the *Arabidopsis thaliana* root as a function of time. We found that gene-expression response to nitrate is dynamic and highly localized and identified cell type–specific transcription factor (TF)–target interactions. Among cell types, the endodermis stands out as having the largest and most connected nitrate-regulatory gene network. ABF2 and ABF3 are major hubs for transcriptional responses in the endodermis cell layer. We experimentally validated TF–target interactions for ABF2 and ABF3 by chromatin immunoprecipitation followed by sequencing and a cell-based system to detect TF regulation genome-wide. Validated targets of ABF2 and ABF3 account for more than 50% of the nitrate-responsive transcriptome in the endodermis. Moreover, ABF2 and ABF3 are involved in nitrate-induced lateral root growth. We obtained an unprecedented spatiotemporal resolution of the root response to nitrate and identified important components of cell-specific gene regulatory networks.

**Key words:** nitrate, regulatory networks, ABF2, ABF3, cell-type, Arabidopsis, root development

# GENOMES UNCOUPLED1-dependent biogenic thermal stress responses

Chaojun Cui[1,2], Shan Qi[1,2], Jieya Xia[1,2], **Chanhong Kim**[1,2]

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**Abstract**

Genomes Uncoupled1 (GUN1), a nuclear-encoded chloroplast pentatricopeptide repeat (PPR) protein, mediates chloroplast-to-nucleus retrograde signaling as a master integrator of diverse retrograde signals. Although PPR proteins primarily function in organelle RNA metabolism, a target RNA of GUN1 remained unknown. This study reveals that GUN1 recognizes a *psbD* transcript derived from a blue light-responsive promoter (BLRP), a transcript referred to as *psbD* BLRP. While overexpression of GUN1 significantly decreases the level of *psbD* BLRP, the loss of GUN1 leads to the accumulation of the putative target RNA. The *in vitro* RNA and *in vivo* genetic studies further reveal the critical role of the C-terminal small MutS-related (SMR) domain in stimulating *psbD* BLRP processing and PsbD (D2, a PSII core protein) synthesis, promoting PSII biogenesis during the early seedling development and de-etiolation. Notably, GUN1 protein also confers seedlings' thermotolerance upon germination with the critical function of the SMR domain. Here, we will discuss the latent function of GUN1 in thermotolerance as an RNA-binding protein and a key regulator of biogenic retrograde signaling.

**Key words:** GUN1, Retrograde Signaling, RNA, *psbD* BLRP, Thermotolerance

# A bi-kinase module sensitizes and potentiates NOX activation in systemic immune signaling

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**Abstract**

Systemic signaling is an essential hallmark of multicellular life. Pathogen encounter occurs locally but triggers organ-scale and organismic immune responses. In plants, elicitor perception provokes systemically expanding $Ca^{2+}$ and $H_2O_2$ signals conferring immunity. Our work identified a $Ca^{2+}$ sensing bi-kinase module as becoming super-activated through mutual trans-phosphorylation and imposing synergistically enhanced NADPH oxidase activation. A combined two-layer bi-kinase/substrate phospho-code allows for sensitized signaling initiation already by near-resting elevations of $Ca^{2+}$ concentration at the infection site. Subsequently, it facilitates further signal wave proliferation with minimal amplitude requirement, triggering protective defense responses throughout the plant.

Specifically, these findings allow to deduce a model in which in the primary elicitor exposed cells, elicitor binding to PRR complexes activates the RLK BIK1. During defense signaling initiation, BIK1 in turn phosphorylates RBOHD at multiple sites including Ser343/347 resulting in NOX activation and local extracellular ROS production. Paracrine signaling by apoplastic ROS activates $Ca^{2+}$ channels in neighboring cells, allowing for subtle increases in cellular $Ca^{2+}$ concentration, that suffice to activate the CBL1/CIPK26/CPK5 module in the absence of PRR activation. Both kinases synergistically phosphorylate and thereby activate RBOHD for maximal ROS production already triggered through minute elevation of cytoplasmic $Ca^{2+}$ concentration. This allows signal propagation to the next distal cell eventually forming an iterative paracrine cell-to-cell signaling circuit manifesting as propagating $Ca^{2+}$/ROS signal. In this way, the CBL1/CIPK26/CPK5/RBOHD module concomitantly confers organ-scale signaling initially within the primary challenged leaf, but also subsequently throughout the whole plant resulting in systemic immunity.

Collectively, this study reveals how plants build and perpetuate trans-cellular immune signal proliferation while avoiding disturbance of ongoing cellular signaling along the path of response dissemination.

**Key words:** CBL, CIPK, ROS, calcium signaling, *Arabidopsis*

# Arabidopsis PRP6 homolog *STA1*-mediated gene expression is important for thermal stress tolerance

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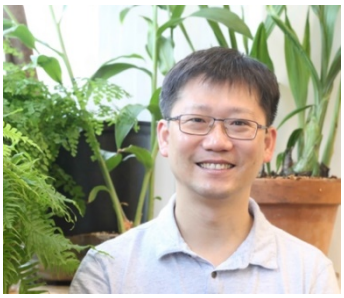
**Abstract**

The Arabidopsis *STABILIZED1* (*STA1*) gene encodes a putative pre-mRNA processing factor. It is homologous to the human U5 snRNP-associated 102-kDa protein (PRPF6) and the yeast pre-mRNA splicing factors PRP1p (fission yeast) and Prp6p (budding yeast). Originally, the *Arabidopsis* mutant defective in the *STA1* gene was isolated during a genetic screen for stress-responsive gene deregulation mutants. The *sta1-1* mutant showed pleiotropic developmental defects and temperature sensitivity. Consistently, *STA1* was expressed in all tissues and is induced by temperature stress. The *sta1-1* mutant also showed a reduced accumulation of miRNAs and defects in pre-mRNA splicing and transcript stability. Two other alleles, *sta1-2* and *sta1-3*, also exhibited phenotypes similar to *sta1-1*, confirming that the defects associated with *sta1-1* were not allele-specific and that *STA1* functions in pre-mRNA splicing, miRNA accumulation, plant growth, and thermal stress tolerance. We overexpressed *STA1* in *Arabidopsis thaliana* and its close relatives *Brassica napus* and *Brassica rapa*, resulting in increased heat tolerance. In addition, we performed a genetic modifier screen of *sta1-1*. Our efforts to understand the function of *STA1* will be presented and discussed.

# Exploring novel regulators in nitrate signaling

**Kun-hsiang Liu**[1,2.]



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**Abstract**

Nitrate is the preferred nitrogen source for most higher plants and not only serves as a macronutrient but also functions as a signaling molecule. Nitrate induces the expression of genes involved in nitrate responses and promotes root elongation and leaf development. However, the mechanisms by which plants perceive nitrate and the molecular processes underlying nitrate signaling remain poorly understood. In a previous study, we discovered that nitrate induces distinct calcium waves, and calcium-dependent protein kinases (CPKs) serve as calcium sensors to transmit nitrate signals. Additionally, our research identified the crucial transcription factor NLP7 as a direct substrate for phosphorylation by CPK10/30/32, which leads to its nuclear retention and subsequent activation of primary nitrate-responsive genes. Notably, we recently established the pivotal role of NLP transcription factors as master regulators in nitrate signaling. Through analysis of NLP-NIN chimeras, we determined that the N-terminus of NLP7 acts as a repressive domain within full-length NLP7, and its repression is alleviated in the presence of nitrate. Moreover, we demonstrated that NLP7 can directly bind to nitrate, functioning as a nitrate sensor. To visualize nitrate binding, we developed a genetically encoded fluorescent biosensor (sCiNiS) for detecting nitrate binding in plants. The nitrate sensor domain of NLP7 shares similarities with the bacterial nitrate sensor NreA, and mutations in conserved residues within the ligand-binding pocket impede the nitrate-triggered function of NLP7.

**Key words:** Nitrate sensor, NLP7, nitrate signaling, *Arabidopsis*

# Cell type-specific responses to fungal infection in plants revealed by single-cell transcriptomics

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**Abstract**

Plant infection by microbial pathogens is a dynamic process but the heterogeneity of plant responses in the context of pathogen localization is poorly understood. We determined the single cell gene expression atlas of *Arabidopsis thaliana* leaf tissue challenged by the fungal pathogen *Colletotrichum higginsianum*. Our results highlight an enrichment of intracellular immune receptors (NLRs) in vasculature-related cells.

Combining pseudotime trajectory inference and live-cell imaging, we were able assign plant cells with a close proximity to the invading fungal hyphae and defined them as infection sites. By separating these cells in major cell types (epidermal, mesophyll, guard and vasculature cells), we found gene expression changes with a strong cell type-specificity. For example, a transcriptional reprograming of abscisic acid signalling was specifically activated in guard cells at the infection sites. Consistent with this pattern, we observed gradual stomatal closure in corelation to their proximity to the fungal invasive hyphae. This may represent a plant defense response in anticipation of pathogen penetration. In addition, we identified genes modulating glucosinolate biosynthesis that were activated in both epidermal and mesophyll cells at the infection sites. However, different sets of genes regulating glucosinolate biosynthesis were employed in epidermal vs mesophyll cells. We then experimentally confirmed that the transcription factor MYB122 was specifically induced in epidermal cells at the infection sites and contributed to defense against fungal infection. Our research highlights the spatiotemporal dynamics of plant response during fungal infection and revealed previously unknown cell type-specific processes and gene functions.

**Key words:** Host-pathogen interaction, single-cell RNA-seq, plant immunity, fungal infection, cell type-specific gene expression, NLR, *Arabidopsis*

# 2D-phase separation for plant signaling

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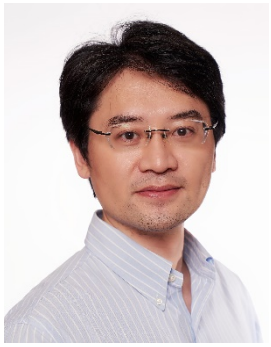

**Abstract**

Our understanding of cellular processes has been greatly enriched by the discovery of membrane-bound and membranous organelles, particularly their role in regulating numerous signal transduction pathways. One such regulatory mechanism is the two-dimensional phase separation (2D-PS), which occurs on the surface of the plasma membrane or intracellular membrane compartments. This presentation will focus on the plasma membrane-based condensation at the interface between microbes and their host plant. Our research aims to characterize and understand the function of phase separation in regulating immune regulatory factors during pathogenic infection on the plant surface. Through the use of biophysics, cell biology, and computational approaches, we will study the spatiotemporal regulation of the condensation process, as well as the material properties and biochemical activities of the resulting condensates. By investigating the underlying mechanisms by which condensation modulates plant immune responses, we aim to contribute to a fundamental understanding of immune signaling during plant-pathogen interactions. Additionally, we will explore how mechano-forces regulate condensation and function by integrating with the plant cell wall-plasma membrane-actin cytoskeleton continuum at the frontline of defense. Our goal is to utilize cutting-edge technologies to inform new strategies for enhancing plant immunity by contributing to a better understanding of the mechanisms involved in immune signaling during plant-pathogen interactions at their interface.

**Key words:** Phase Separation, Host-Microbe Interaction, Plant Immunity, Mechanoregulatoin, Actin Remodeling

# Inactivation of rice *lov kelch repeat protein 2* enhances drought tolerance by increasing cuticular wax biosynthesis

**Nam-Chon Paek**[1,2]

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*Correspondence: ncpaek@snu.ac.kr

**Abstract**

Drought tolerance is important for grain crops, including rice (*Oryza sativa*); for example, rice cultivated under intermittent irrigation produces less methane gas compared to rice grown in anaerobic paddy field conditions, but these plants require greater drought tolerance. Moreover, the roles of rice circadian-clock genes in drought tolerance remain largely unknown. Here, we show that the mutation of *LOV KELCH REPEAT PROTEIN 2* (*OsLKP2*) enhanced drought tolerance by increasing cuticular wax biosynthesis. Among *ZEITLUPE* family genes, *OsLKP2* expression specifically increased under dehydration stress. *OsLKP2* knockdown (*oslkp2-1*) and knockout (*oslkp2-2*) mutants exhibited enhanced drought tolerance. Cuticular waxes inhibit non-stomatal water loss. Under drought conditions, total wax loads on the leaf surface increased by approximately 10% in *oslkp2-1* and *oslkp2-2* compared to the wild type, and the transcript levels of cuticular wax biosynthesis genes were upregulated in the *oslkp2* mutants. Yeast two-hybrid, bimolecular fluorescence complementation, and co-immunoprecipitation assays revealed that OsLKP2 interacts with GIGANTEA (OsGI) in the nucleus. The *osgi* mutants also showed enhanced tolerance to drought stress, with a high density of wax crystals on their leaf surface. These results demonstrate that the OsLKP2-OsGI interaction negatively regulates wax accumulation on leaf surfaces, thereby decreasing rice resilience to drought stress.

**Key words:** OsLKP2, OsGI, drought tolerance, cuticular was biosynthesis, rice

# North, south, up and down. The genes and molecules that allow maize to move all around

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**Abstract**

Native Americans living in the southwest of Mexico domesticated maize and brought it to places as far North as the Gaspé peninsula in Canada and as high as the Sacred Valley in Cusco, Perú. During this process, maize adapted to longer days, lower temperatures, and a phenomenal diversity of environments. In this talk, I will present current knowledge about the genetic changes that allowed maize adaptation to this wide array of growing conditions and our recent results on the role of phospholipid metabolism in maize highland adaptation.

# Phytochrome photoactivation and signaling: finally light at the end of the tunnel

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**Abstract**

Most organisms use a collection of photoreceptors to entrain their growth, development, and reproduction with the ambient light environment. One influential class in plants, bacteria, and fungi are the phytochromes (Phys), a family of dimeric bilin-bound photoreceptors responsible for controlling a wide array of signaling outcomes by reversibly transitioning from a dark-adapted Pr state to a red light-activated Pfr state. While our understandings of the downstream events are emerging, it is still unclear how Phys convert light absorption into a conformational signal. In the past decade we have employed structure-based approaches to define these early events using tractable bacterial and plant models. By X-ray crystallography of the photosensory module (PSM) harboring the bilin within its GAF domain, we discovered that key events in bacterial Phys are a β-stranded to α-helical transition of a unique anti-parallel hairpin loop emanating from the PHY domain to contact the GAF domain. This transition displaces a helical spine connecting the GAF and PHY domains, with the resulting torsional strain then translating into the downstream output module (OM). Often, the OM includes a two-component histidine kinase domain, which allows microbial Phys to initiate light-regulated phosphotransfer cascades. Then by exploiting serial femtosecond and temperature scanning crystallography using a Phy that permits photointerconversion even in a crystal lattice, we found that one of the earliest events is a rotation of the bilin D-pyrrole, which is driven by a light-induced isomerization of the C15=C16 methine bridge.

Several structures of full-length microbial Phy dimers revealed that they assemble head-to-head using contact sites within the paired PSMs and OMs. Surprisingly, our recent atomic-resolution cryo-EM structures of Arabidopsis PhyA revealed an unanticipated 3D architecture for plant Phys that likely provides a unique signaling mechanism. While the C-terminal end includes a histidine-kinase-related (HKR) sequence that retains its head-to-head orientation, the PSMs are no longer in contact and instead are arranged head-to-tail with an intervening modulator loop and PAS domain connecting the two PSMs to form an extensive parallelogram-shaped platform. Mutational analyses revealed that these modulator/PAS contact sites are critical for Pfr stability: weakening

these contact sites not only destabilizes dimerization, they also dampen the rate of Pfr→Pr thermal reversion. As this platform would be impacted by the same hairpin reconfigurations during photoconversion, we presume that it provides contact sites for binding to various downstream partners that are genetically linked to photoperception. Intriguingly, the HKR domains rise above the platform asymmetrically, which might differentially influence signaling and stability of the Pfr state. Besides PhyB, plants typically express a family of Phy isoforms with overlapping and unique roles in photoperception that are likely underpinned by unique biophysical properties. For example, while PhyB reverts quickly from Pfr to Pr and is most influential in full sun environments as experienced by mature plants, the PhyA isoform is stable as Pfr and is more influential under dim light as experienced by germinating seeds and seedling emerging from the soil. To understand these structural differences, we recently generated a 3D structure of Arabidopsis PhyA, and found unique structural properties that likely induce its unique signaling potential. Collectively, the Arabidopsis Phy structures highlight how these photoreceptors diversified to extend light and temperature perception in plants.

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Burgie*, E.S., H. Li*, Z.T.K. Gannam*, K. McLoughlin, **R.D. Vierstra**, and H. Li (2023) Structure of *Arabidopsis* phytochrome A reveals topological and functional diversification among the plant photoreceptor isoforms. *Nature Plants* doi.org/10.1038/s41447-023-01435-8. (*co-first authors)

# From single cells to multicellular life and vice versa

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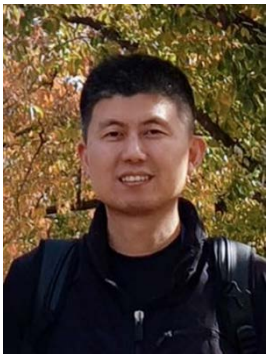

**Abstract**

The emergence of multicellular organisms, including plants and animals, marks a significant evolutionary milestone in the history of life. Despite their diverse forms and functions, all multicellular creatures trace their origins back to single-cell organisms. Unravelling the drivers and mechanisms that led to the evolution of multicellularity presents inherent challenges, as this transition occurred hundreds of millions of years ago and the ancestral single-celled species no longer exist.

To comprehend the shift from single-celled to multicellular organisms, a fundamental aspect to explore is the division of labor among the different cells comprising each multicellular organism. In recent years, it has become clear that variations in gene expression at the single-cell levels contribute to the remarkable diversity observed among cells. Advancements in single-cell transcriptomics have provided an opportunity to quantitatively and comprehensively analyze these intricate gene expression patterns. By understanding how cell type- and state-specific gene expression programs are established under varying environmental conditions, including stressful ones, we can gain insights into the evolutionary processes underlying the development of multicellularity and the conditions that facilitated its emergence.

One intriguing question pertains to the role of specific genes responsible for cellular processes such as cell polarity, oriented cell division, and cell-cell adhesion in the context of multicellularity. While these genes are present in single-celled species, it remains a mystery whether and how they are employed during the establishment of multicellular systems. Did the horizontal transfer and duplication of these genes, along with the selection and expansion of new gene functions, play a pivotal role in orchestrating the physical and functional integration of cells into a coherent multicellular entity? By addressing these questions, we aim to shed light on the fascinating journey from unicellularity to multicellularity and vice versa, further enriching our understanding of life's remarkable complexity.

**Keywords:** unicellularity, multicellularity, cell-cell adhesion, single cell transcriptomics, environmental conditions

# Small G protein signaling: bridging the gap between pattern formation and abiotic stress

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**Abstract**

ROP small G proteins in plants have primarily been investigated for their role in regulating cell growth. Interestingly, there exists a mutual antagonism between ROPs and ABA signaling, indicating that ROPs operate at the intersection of cell growth and abiotic stress responses. During my presentation, I will elucidate our research conducted on two model systems: guard cell signaling in tomato and the differentiation and patterning of the secondary cell wall in the primary xylem of Arabidopsis roots. Our investigations have unveiled both cell autonomous and non-cell autonomous interactions between ABA and ROP signaling encompassing positive and negative feedback loops. Furthermore, I will discuss the outcomes of a field experiment and their consequential implications.

# The HOS15-HDA9 complex associates with HYL1 to modulate miRNA expression in response to ABA signaling

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**Abstract**

The regulation of microRNAs (miRNAs) biogenesis in plants is crucial for maintaining plant homeostasis under biotic and abiotic stress. The crosstalk between the RNA polymerase II (Pol-II) complex and miRNA processing machinery has emerged as a central hub modulating transcription and co-transcriptional processing of pri-miRNAs. However, it remains unclear how miRNA-specific transcriptional regulators recognize *MIRNA* loci. Here, we show that the HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 15 (HOS15)/HISTONE DEACETYLASE 9 (HDA9) complex is a conditional suppressor of miRNA biogenesis particularly in response to ABA. Under ABA-treatment, *hos15/hda9* mutants present an enhanced pri-miRNA transcription that is balanced with an increased processing, leading to over-accumulation of a set of mature miRNAs. Moreover, the ABA-induced recruitment of the HOS15-HDA9 complex to *MIRNA* loci is guided by HYL1 upon recognition of the nascent pri-miRNAs. The HYL1-dependant recruitment of HOS15-HDA9 complex to *MIRNA* loci suppresses *MIRNA* expressions and pri-miRNA processing. Most importantly, our findings indicate that nascent pri-miRNAs serve as scaffolds for recruiting transcriptional regulators specifically to *MIRNA* loci. This discovery indicates that RNA molecules can act as regulators of their own expression, for example causing a feedback loop that turn off their transcription, perhaps in a self-buffering system.

**Key words:** miRNA, HOS15, HYL1, transcriptional regulation, *Arabidopsis*

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