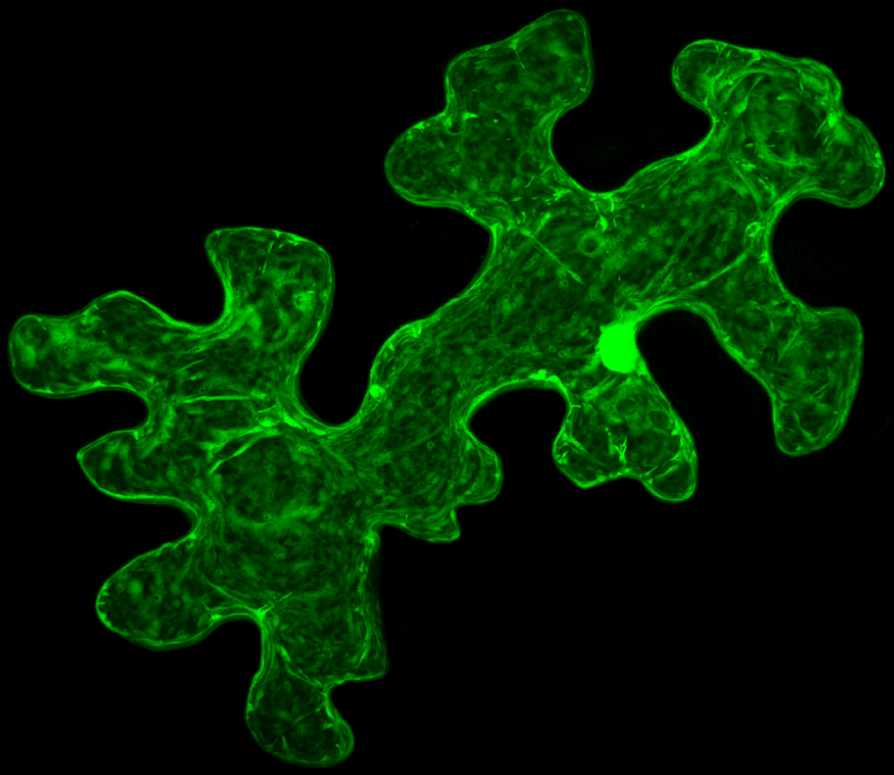
Second Plant Cell Atlas Symposium

Program Book

December 12 & 13, 2022



ACKNOWLEDGEMENTS

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Benjamin Cole (Lawrence Berkeley National Laboratory, USA)
Margaret Frank (Cornell University, USA)
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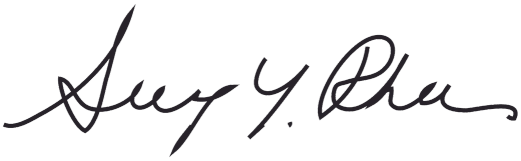
CARNEGIE SCIENCE

2022 Plant Cell Atlas Symposium

The Plant Cell Atlas (PCA) 2022 Symposium will share exciting scientific and technological breakthroughs in plant science, create a forum for people interested in these approaches and their application to plants to meet and network, discuss the needs of the PCA community, and gather input on what is needed to develop a Plant Cell Atlas resource.

The Plant Cell Atlas is an international community, and the 2022 Symposium is bringing together researchers from all around the world. **To make the Symposium accessible in many different time zones, the oral and posters sessions are spread throughout the day, and there are extended breaks in the schedule. Please review the conference overview on page 5.**



Principle Investigator
PCA Research Coordination Network

The 2022 Plant Cell Atlas (PCA) Symposium will also serve as a space to network and discuss the vision of the PCA. Join us for the community discussions.

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9:20 AM - 10:20 AM PST	**Poster Session One**
10:20 AM - 3:00 PM PST	***Mid-day Recess***
3:00 PM - 5:10 PM PST	**Oral Session Two**

DAY TWO * DECEMBER 13TH

7:00 AM - 9:10 AM PST	**Oral Session Three**
9:10 AM - 3:00 PM PST	***Mid-day Recess***
3:00 PM - 5:10 PM PST	**Oral Session Four**
5:10 PM - 5:20 PM PST	**Break**
5:20 PM - 6:20 PM PST	**Poster Session Two**

To make the Symposium accessible in many different time zones, the oral and posters sessions are spread throughout the day. Each day there is an **extended break** in the schedule.

ORAL SESSION ONE

Dec 12th, 2022

AGENDA

7:00 AM - 7:05 AM PST	Introduction **Moderators: Devang Mehta and Kaisa Kajala**
7:05 AM - 7:25 AM PST	Transcriptional dynamics underlying cell fate specification in the Arabidopsis root **Rachel Shahan, Duke University, USA**
7:35 AM - 7:45 AM PST	Discovery of conserved marker genes for cross-species cell type assignment by machine learning **Tran Chau, Virginia Polytechnic Institute, USA**
7:50 AM - 8:10 AM PST	Technology advances in single-cell and spatial proteomics for plant biology **Ying Zhu, Genentech, USA**
8:20 AM - 8:30 AM PST	Gene network discovery and engineering to enhance rice root resilience **Alexander Borowsky, University of California, Riverside, USA**
8:35 AM - 8:40 AM PST	Transition to Community Discussion 1 **Moderators: Devang Mehta and Kaisa Kajala**
8:40 AM - 9:10 AM PST	Community Discussion 1: "Major challenges/bottlenecks and outstanding questions for single cell omics" **Moderators: Devang Mehta and Kaisa Kajala**
9:10 AM - 9:20 AM PST	Break
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Abstracts for the talks are available on pages 12-28

POSTER SESSION ONE

Dec 12th, 2022

POSTER SESSION 1A (9:20 AM - 9:35 AM PST)

Poster #1: "Large scale rice transcriptome analysis reveals unique pathways and defense genes involved in rice-Xanthomonas interaction" by **Kamal Kumar Malukani, Tata Institute for Genetics and Society, Bangalore, India**

Poster #2: "Phosphorylation Status of B beta Subunit Acts as a Switch to Regulate the Function of Phosphatase PP2A in Ethylene-mediated Root Growth Inhibition" by **Zhengyao Shao, University of Texas at Austin, USA**

Poster #3: "Separation of cork and vascular cambia identities from a single cell" by **Jennifer López Ortiz, University of Helsinki, Finland**

Poster #4: "Non-negative matrix factorization (NMF) is applied to infer cellular composition and constituent gene expression programs (GEPs) from scRNAseq data" by **Yasir Arafat Tamal, Max Planck Institute for Plant Breeding Research, Germany**

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Poster #5: "Comparative *in silico* analysis of the Haloacid Dehalogenase PS2-like enzymes in *Capsicum annum* and *Arabidopsis thaliana*" by **Rogelio Rodríguez-Sotres, Universidad Nacional Autónoma de México, México**

Poster #6: "Spatiotemporal Dynamics Of Cell Plate Development During Plant Cytokinesis" by **Rosalie Sinclair, University of California, Davis, USA**

POSTER SESSION 1C (9:50 AM - 10:05 AM PST)

Poster #7: "Single-cell analysis of plant shoot meristems opens a 'goldmine' for functional studies" by **Xiaosa Xu, Cold Spring Harbor Laboratory, USA**

Poster #8: "A Receptor-Like Protein PAN2 is required for ABA and dark-mediated grass stomatal closure via its expression on the subsidiary cell plasma membrane" by **Le Liu, University of Massachusetts, USA**

Poster #9: "The purple acid phosphatase PAP26 is essential for efficient nucleotide metabolism in the vacuole of Arabidopsis" by **Ang-Yu Liu, Iowa State University, USA**

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Poster #11: "Spatial reconstruction of single-cell gene expression in floral meristems of *Arabidopsis thaliana* " by **Manuel Neumann, Humboldt University Berlin, Germany**

Poster #12: "The nucleus decides the future division site" by **Arif Ashraf, University of Massachusetts Amherst, USA**

Posters in the same session will be presented concurrently. Abstracts are available on pages 28-54

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Dec 12th, 2022

AGENDA

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3:35 PM - 3:45 PM PST	Examining arbuscular mycorrhizal symbiosis using spatial transcriptomics in *Medicago truncatula* **Trevor Tivey, Boyce Thompson Institute, USA**
3:50 PM - 4:10 PM PST	Deep learning for genomic discovery **Anshul Kundaje, Stanford University, USA**
4:20 PM - 4:30 PM PST	*In situ* spatial metabolomics for revealing root-microbe molecular interactions down to the cellular scale **Dušan Veličković, Pacific Northwest National Laboratory, USA**
4:35 PM - 4:40 PM PST	Transition to Community Discussion 2 **Moderator: Benjamin Cole**
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Abstracts for the talks are available on pages 12-28

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Dec 13th, 2022

AGENDA

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3:50 PM - 4:10 PM PST	Leaf epidermal patterning and fate determination **Chin-Min Kimmy Ho, Academia Sinica, Taiwan, R.O.C.**
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4:40 PM - 5:10 PM PST	Community Discussion 4: "How can we navigate the evolving social media to promote the work and people of the PCA community?" **Moderator: Chris Anderton**
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Dec 13th, 2022

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Poster #14: "Secretory peptides of the bryophyte-specific protein SHORT-LEAF regulate gametophore development in moss *P. patens* " by **Shirsa Palit, Indian Institute of Science Education and Research (IISER), India**

Poster #15: "Single-cell genomics and high-throughput phenotyping for determining the quantitative genetics of maize leaf vascular development " by **Diana Ruggiero, Oregon State University, USA**

Poster #16: "A single-nucleus transcriptome atlas of seed-to-seed development in Arabidopsis " by **Travis Lee, Salk Institute for Biological Studies, USA**

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Poster #18: "Slow and not so furious: *de novo* stomatal pattern formation during plant embryogenesis" by **Margot Smit, Stanford University, USA**

Poster #19: "A Dof-CLE circuit controls phloem organization" by **Pingping Qian, Osaka University, Japan**

POSTER SESSION 2C (5:50 PM - 6:05 PM PST)

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Poster #21: "Unbiased RNA and protein co-expression networks highlight important regulatory role of organelle protein homeostasis in maize heterosis" by **Bridget Hua Bai, UC San Diego, USA**

Poster #22: "Tissular and Subcellular Localization of a Protein Induced by Water Deficit in *Arabidopsis Thaliana*: The case of AtLEA4-5 Protein " by **Coral Martínez, Universidad Nacional Autónoma de México, México**

Poster #23: "Mapping multi-kingdom symbiotic interactions with spatial transcriptomics and single-nucleus sequencing" by **Karen Serrano, Joint BioEnergy Institute, USA**

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Poster #25: "Network-enabled regulatory dissection of the mixed-linkage glucan synthase genes in grasses" by **Dae Kwan Ko, Michigan State University, USA**

Poster #26: "Mapping Cell-Specific Activity During Germination Of Barley Grain Using Spatial Transcriptomics" by **Marta Peirats-Llobet, La Trobe University, Australia**

Poster #27: "Cell-type Specific Responses of Poplar to Stress Combination: An Integrated Network Assisted Proteomics and Spatial Metabolomics Approach" by **Vimal Kumar Balasubramanian, Pacific Northwest National Laboratory, USA**

Posters in the same session will be presented concurrently. Abstracts are available on pages 28-54

RACHEL SHAHAN

Duke University, USA

December 12th, 2022

7:05 AM - 7:25 AM PST

Dr. Shahan is a Ruth L. Kirschstein NRSA Postdoctoral Fellow in the Benfey Lab at Duke University. She uses the plant root as a tractable model to study gene expression changes underlying tissue differentiation.

Transcriptional dynamics underlying cell fate specification in the Arabidopsis root

Spatiotemporal gene regulatory networks orchestrate organ development. Mapping global gene expression patterns across developmental time is essential to understand how these networks facilitate tissue patterning, cell identity acquisition, and terminal tissue differentiation. To this end, we combined single cell RNA-sequencing (scRNA-seq) data from over 110,000 cells to construct a gene expression atlas of the Arabidopsis root. The atlas profiles the specification of each cell fate and suggests that differentiation is underpinned by gradual transcriptional changes rather than a series of switches. We applied stationary optimal transport, a variant of Waddington Optimal Transport (Schiebinger et al. 2019) for systems in equilibrium, to reconstruct comprehensive developmental trajectories for each cell type and identify candidate regulators of differentiation. To test the utility of the atlas to interpret smaller datasets, we profiled mutants of two key transcriptional regulators at single cell resolution, *shortroot* and *scarecrow*. In addition to reflecting known tissue composition phenotypes for both mutants, the data suggest a previously unappreciated cell identity transition in the scarecrow mutant. With the atlas annotation as a resource, we are continuing to use scRNA-seq as a powerful tool to probe cell identity phenotypes in developmental mutants. Most recently, we used time-series scRNA-seq to profile Arabidopsis root responses to brassinosteroids (Nolan et al., 2022) and we are currently investigating the transcriptional dynamics underlying root development as seedlings age. To streamline the scRNA-seq data processing workflow and provide an accessible point of entry for first time users, we have also developed a comprehensive pipeline that can be applied to data from any species, organ, or tissue (Hsu et al., 2022).

TRAN CHAU

Virginia Polytechnic Institute, USA

December 12th, 2022

7:35 AM - 7:45 AM PST

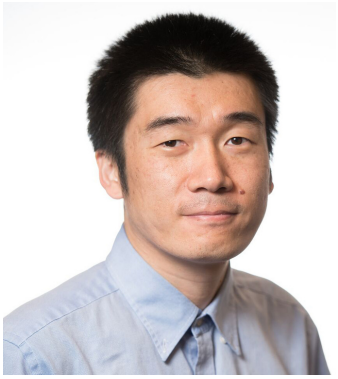
Tran Chau is a Ph.D. student in the Song Li lab at the Virginia Polytechnic Institute. She applies machine learning methods to solve problems related to single-cell transcriptomic data across plant species.

Discovery of conserved marker genes for cross-species cell type assignment by machine learning

Tran Chau (1,2), Prakash Raj Timilsina (1), Song Li (1,2)

1: School of Plant and Environmental Sciences, Virginia Tech.
2: Graduate Program in Genetics, Bioinformatics, and Computational Biology. Virginia Tech.

The use of single-cell RNA sequencing (scRNA-seq) technology in plant research and crop improvement has been gaining traction. The gene expression profile from scRNA-seq can provide new insights into the conservation and divergence of cell types and holds the promise of transferring the knowledge of cell type-specific molecular functions across plant species. However, the lack of known cell type marker genes in non-model species, and the complexity of gene homology among plants present hindrances in cell type identification in plant single-cell data. The goal of this study was to determine conserved marker gene sets from all root cell types using published scRNA-seq data of Arabidopsis, maize (*Zea mays*), and rice (*Oryza sativa*). To this end, we applied differential expression analysis based on the Wilcoxon rank sum test in the Seurat package to identify marker genes. We also applied two feature selection techniques: (1) identification of SHAP markers using random forest and (2) identification of SVM markers using support vector machines. To overcome the challenge of gene homology, we utilized OrthoFinder to map species marker genes onto orthogroup markers (OGM), which are a set of marker genes derived from a common ancestral gene. By leveraging single-cell transcriptomics and applying these machine-learning methods, our analysis identified small but conserved cell-type orthologous genes across monocot and dicot species. For example, among the top 200 most significant marker genes in xylem cells of each species, there are 12, 18, and 14 OGMs identified by Seurat, SHAP, and SVM respectively. We have found a higher number of orthologous genes in the xylem and cortex than in other cell-type clusters. Interestingly, while the endodermis clusters in rice and maize do not share any common OGMs with Arabidopsis, we have found 65 and 57 endodermis OGMs that are marker genes for stele and atrichoblast clusters in Arabidopsis. This result suggests the existence of functional conservation as well as substantial divergence between these cell types. Taken together, we expect these orthologous gene lists will be useful in determining the cell types in non-model plants and will open the opportunity for facilitating cell type annotations across plant species in the future.



YING ZHU

Genentech, USA

December 12th, 2022

7:50 AM - 8:10 AM PST

Dr. Zhu is Senior Principal Scientist at Genentech. His research focuses on developing highly sensitive/ high-throughput technologies for single cell and spatial proteomics.

Technology advances in single-cell and spatial proteomics for plant biology

Although mass spectrometry–based proteomics has been demonstrated as an enabling discovery tool for studying plant physiology, conventional approaches require millions of cells to generate useful biological conclusions. Such requirements mask the cell-to-cell heterogeneities and limit the comprehensive profiling of plant proteins at spatially resolved and cell-type-specific manner. In this talk, I will give a brief introduction of our effort to push the boundaries of proteomics to single-cell and spatially resolved measurement. I will introduce nanoPOTS, a microfluidics-based sample preparation method to allow highly efficient protein processing for mass spectrometry measurements. New developments including high throughput spatial proteomics and single-cell multiomics will be described. I will also show several ongoing collaborations with plant biologists to apply the technologies to understand the cell-type-specific response to abiotic stress in different model plant systems.

ALEXANDER BOROWSKY

University of California, Riverside, USA

December 12th, 2022

8:20 AM - 8:30 AM PST

Alex Borowsky is a NIFA Predoctoral Fellow in Dr. Julia Bailey-Serres' lab at University of California, Riverside, where he studies rice resilience to abiotic stress using computational, genetic, and synthetic biology approaches.

Gene network discovery and engineering to enhance rice root resilience

Alex Borowsky, Mauricio Reynoso, Julia Bailey-Serres

Center For Plant Cell Biology (CEPCEB)

Understanding how roots modulate development and metabolism under varied irrigation or rainfall is crucial for development of climate resilient crops. However, root development and environmental response involves the complex orchestration of different genetic programs in different cell types. For example, in rice, a water- and airtight barrier of suberin in the exodermis is formed in response to drought and waterlogging. We established a toolbox of tagged rice lines to profile translating mRNAs and chromatin accessibility within specific cell populations. We used these tools to generate multi-omic profiles of rice root cell types in a range of environments: plates in the lab, controlled greenhouse stress and recovery conditions, and outdoors in a paddy. Through integration of chromatin and mRNA data, we resolve regulatory networks of genes involved in the drought-responsive deposition of suberin in the exodermis. Using this information, we are using engineering and synthetic biology approaches to manipulate the levels of suberin in the exodermis, both under stress and in well-watered conditions. Ultimately, we hope that these engineered plants will demonstrate enhanced tolerance to multiple stresses.

TATSUYA NOBORI

Salk Institute for Biological Studies, USA

December 12th, 2022

3:05 PM - 3:25 PM PST

Dr. Nobori is an HFSP Postdoctoral Fellow at Salk Institute for Biological Studies. His research aims to understand plant-microbe interactions at single-cell resolution by incorporating/developing new sequencing & imaging technologies.

PHYTOMap: Multiplexed single-cell 3D spatial gene expression analysis in plant tissue

Understanding how individual cells respond and interact with each other in the face of changing environments is the cornerstone of understanding tissue function. The increasing throughput and sensitivity in single-cell transcriptomics technologies are offering tremendous granularity at which cells can be classified, but it also creates new challenges in dealing with cell populations that our current histological and physiological understanding of plant cells cannot account for. To understand the identity and function of molecularly defined cell populations, it is critical to analyze their spatial localization and complex responses in the native three-dimensional tissue context. Here, we present PHYTOMap (Plant HYbridization-based Targeted Observation of gene expression Map), a multiplexed fluorescence *in situ* hybridization method that enables single-cell and spatial analysis of gene expression in whole-mount plant tissue. We applied PHYTOMap to simultaneously analyze 28 cell-type marker genes in Arabidopsis roots and successfully identified major cell types, demonstrating that our method can substantially accelerate the spatial mapping of marker genes defined in single-cell RNA-seq datasets in complex plant tissue. PHYTOMap can be performed in a standard molecular biology lab with a confocal microscope at a low cost. The transgene-free nature of PHYTOMap makes this technology potentially applicable to any plant species. Cell type annotation in scRNA-seq is challenging in many crop plants as their marker genes are often not conserved in other well-characterized species such as Arabidopsis. We envision that PHYTOMap can facilitate efficient cluster annotation in scRNA-seq studies of a variety of plant species. Beyond cell typing, PHYTOMap will offer unique opportunities to interrogate spatial regulation of complex cellular responses in plant tissue during stress and development.

TREVOR TIVEY

Boyce Thompson Institute, USA

December 12th, 2022

3:35 PM - 3:45 PM PST

Trevor Tivey is an NSF Postdoctoral Fellow in the Harrison Lab at the Boyce Thompson Institute. Dr. Tivey studies the spatial landscape of nutritional symbioses, currently working on spatial transcriptomics of arbuscular mycorrhizae.

Examining arbuscular mycorrhizal symbiosis using spatial transcriptomics in *Medicago truncatula*

Trevor R. Tivey (1), Iwijn De Vlaminck (2), Maria J. Harrison (1)

1: Boyce Thompson Institute, Ithaca, NY
2: Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY

An arbuscular mycorrhiza (AM) is a symbiotic association that forms between host plant roots and soil fungi. In this underground relationship, fungi penetrate the plant root epidermis and form temporary structures known as arbuscules within plant root cortical cells, resulting in a linear gradient of fungal colonization. The colonization of these plant cells enables both plants and fungi to exchange and acquire critical nutrients. Though many plant genes central to the symbiotic program have been identified and functionally characterized, the broader transcriptional landscape of AM colonization requires further characterization. To detect differences between colonized and noncolonized plant roots, we adopted a spatial transcriptomic approach with the objective to map the plant and fungal transcriptomes along a spatiotemporal gradient of colonization. *Medicago truncatula* roots colonized with the AM fungus *Rhizophagus irregularis* were optimized for spatial transcriptomic procedures, including cryopreservation, fixation, staining, imaging, permeabilization, and enzyme removal. The following 10X Visium gene expression experiment resulted in transcriptomic libraries from four sample sections containing longitudinal and cross-sections of colonized and noncolonized plant roots. Spatial mapping of colonized and noncolonized plant roots revealed clear differences between the transcriptomes of colonized and noncolonized roots, including strong differential expression of both plant and fungal transcripts.

ANSHUL KUNDAJE

Stanford University, USA

December 12th, 2022

3:50 PM - 4:10 PM PST

Dr. Kundaje is an Assistant Professor of Genetics and Computer Science at Stanford University. The Kundaje lab uses deep learning models to decipher the sequence code of regulatory elements and networks, and to predict functional non-coding genetic variation.

Deep learning for genomic discovery

The human genome contains the fundamental code that defines the identity and function of all the cell types and tissues in the human body. Genes are functional sequence units that encode for proteins. But protein coding regions account for just about 2% of the 3 billion long human genome sequence. What does the rest of the genome encode? How is gene activity controlled in each cell type? Where do the regulatory control elements lie and what is their sequence composition? How do variants and mutations in the genome sequence affect cellular function and disease? These are fundamental questions that remain largely unanswered. The regulatory code that controls gene activity is encoded in the DNA sequence of millions of cell type specific regulatory DNA elements in the form of functional DNA words with complex syntax. This regulatory code has remained largely elusive despite tremendous developments in experimental techniques to profile molecular properties of regulatory DNA. We have developed deep learning frameworks to learn how genomic sequence encodes millions of experimentally measured regulatory genomic events across 100s of cell types and tissues. We have developed novel methods to interpret our models and extract local and global predictive patterns revealing many insights into the syntax and grammar of the regulatory code. Our models also serve as *in-silico* oracles to predict the effects of natural and disease-associated genetic variation i.e. how differences in DNA sequence across healthy and diseased individuals are likely to affect molecular mechanisms associated with common and rare diseases. These models enable optimized design of genome perturbation approaches to decipher functional properties of DNA and variants and serve as a powerful lens for genomic discovery.

DUŠAN VELIČKOVIĆ

Pacific Northwest National Laboratory, USA

December 12th, 2022

4:20 PM - 4:30 PM PST

Dr. Veličković is a mass spectrometry imaging scientist at Pacific Northwest National Laboratory with expertise in spatial metabolomics, lipidomics, and glycomics in plant organs, microbial colonies and plant-microbe interfaces.

In situ **spatial metabolomics for revealing root- microbe molecular interactions down to the cellular scale**

Dušan Veličković, Kevin Zemaitis, Mowei Zhou, Christopher Anderton

Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA, 99354, USA

Root surfaces are major sites of interactions between plants and associated microorganisms. Studying the small-scale gradients of biomolecules in these interactions is key for understanding the molecular interplay that regulate these associations. The Environmental Molecular Sciences Laboratory, a U.S. Department of Energy User Facility, has a suite of mass spectrometry imaging (MSI) tools able to address a variety of root and rhizosphere-related questions. Herein, we will present advancements in matrix assisted laser desorption/ionization (MALDI)-MSI for untargeted spatial analysis of plant-microbe molecular interactions. We used our novel approach, imprinting MALDI-MSI to map and identify compounds on the root surface and presumed root exudates and microbial metabolites in the rhizosphere, where hot spots of some organic compounds were observed in distinct pockets around the root. By employing molecular tomography (i.e., three-dimensional MALDI-MSI), we have also been able to determine that metabolic asymmetry exists within soybean root nodules as a function of the plant's symbiosis with rhizobia capable of performing biological nitrogen fixation (BNF). In the same symbiotic system, we were able to show cellular heterogeneity of small proteins, *N*-glycans and specific glycoproteins and hint on novel functional role of *N*-glycosylation in plants, namely establishing an environment for efficient BNF by rhizobia. In mycorrhization, MALDI-MSI provided us insight in relocation of alkaloids during compatible and incompatible plant-fungi interactions and based on spatial colocalization studies, we proposed some alternative routes for biosynthesis of these alkaloids. Lastly, we will also demonstrate some new derivatization protocols that have promise in MALDI-MSI of plant phytohormones, as they have key roles in the interaction between plants and beneficial microbes, yet their spatial interplay remains quite elusive.

TERRI LONG

North Carolina State University, USA

December 13th, 2022

7:05 AM - 7:25 AM PST

Dr. Long is an Associate Professor at North Carolina State University and Education and Workforce Development Platform Director of the N.C. Plant Sciences Initiative. The Long lab currently focuses on regulatory mechanisms that control plant stress response and has discovered several regulatory proteins that play a key role.

How *Iron*ic: Iron at the intersection of plant development and multi-stress resilience

Iron (Fe) is an essential micronutrient that plays critical roles in central metabolic plant processes such as photosynthesis and respiration. The mechanisms by which plants maintain Fe homeostasis are particularly intriguing. While it is relatively abundant, in most soils Fe is insoluble and therefore of limited bioavailability, however excess Fe accumulation in plants can lead to cellular damage. Thus, plants must extract sufficient Fe from recalcitrant growth environments, while also ensuring that Fe content does not exceed a specific range. Arabidopsis and other dicots have developed mechanisms to sense Fe deficiency in the shoot, which triggers roots to solubilize, reduce and uptake Fe across multiple root cell types before transport to the shoot. We have uncovered several molecular mechanisms that control how plants recognize and respond to iron deficiency stress and found new evidence for how specific cell types within the root are involved in these processes. Considering how critical Fe is for overall plant health, it is no surprise that these mechanisms also impact responses to a range of other abiotic and biotic stress conditions.

MARIA SAVINA

Radboud University, The Netherlands

December 13th, 2022

7:35 AM - 7:45 AM PST

Maria Savina is a Postdoc in Plant Systems Physiology at Radboud University. She is interested in genetic mechanisms of plant morphogenesis, and is working to develop a multiscale digital model of auxin distribution in the plant root.

PlantLayout: the platform for mathematical modeling of plant developmental processes within realistic layouts

Maria Savina (1) and Victoria Mironova (1,2)

1 Plant Systems Physiology, Radboud University, Nijmegen, The Netherlands
2 Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

The functioning of any multicellular organism requires the maintenance of the meristems that contain the pool of stem cells throughout the life cycle. One of the most important tasks of developmental biology is to study the mechanisms of stem cell niche maintenance and other processes in the meristems. It is also known that the nature of the effects of hormones and other low-molecular substances depends on the distribution of their concentrations in tissues. Auxin is the most important morphogenic trigger that drives both cell-type-specific intracellular signaling and cell-cell communication in a concentration-dependent manner (Casanova-Sáez et al., 2021). However, there is a problem with monitoring the changes in the auxin concentration itself in each cell during developmental processes in the experiment. Mathematical modeling became an important approach to predicting and understanding auxin's roles at synthesis, signaling, and transport levels during plant development and stress responses (Rutten et al., 2021).

We developed the PlantLayout tool that allows us to make a digital copy of plant organs or tissue and implement mathematical models of hormonal or genetic regulation there. PlantLayout was originally developed for modeling the self-organization of auxin and PIN transporter distribution in the realistic cell layout of the apical root meristem of *Arabidopsis thaliana* (Savina and Mironova, 2020). PlantLayout allows for the development of both individual mathematical models and a series of mathematical models with different cell layouts easily. Moreover, it can be used to model the distribution of any low-molecular substance with known carrier proteins and a certain gene regulatory circuit in any tissue with a known structure.

We used this tool to investigate the mechanism of regeneration of *Arabidopsis thaliana* roots after bleomycin treatment caused the damage using a series of individual cell layouts of damaged roots (Canher et al., 2020). As another application of PlantLayout, we investigated the mechanisms of auxin distribution influence on the haustorium formation process in the parasitic plant *Striga hermonthica* at different developmental stages (Xiao et al., 2022).

A new application of our PlantLayout tool is associated with single-cell sequencing data. We reconstruct “digital expression patterns” based on single-cell RNA sequencing data using a “digital root” created by PlantLayout to represent single-cell data in a more user-friendly way.

CHRISTOPHER ANDERTON

Pacific Northwest National Laboratory, USA

December 13th, 2022

7:50 AM - 8:10 AM PST

Dr. Anderton is a Senior Chemist at the Environmental Molecular Sciences Laboratory at the Pacific Northwest National Laboratory. His research involves visualizing mechanisms driving interkingdom rhizobial interactions to understand drivers of resiliency in a changing environment.

Towards revealing the metabolome within plant tissue at the single cell level with spatially resolved mass spectrometry and mass spectrometry imaging approaches

Christopher Anderton

PNNL, Environmental Molecular Sciences Laboratory

Single-cell omics is a field of increasing interest, as characterizing the transcriptome, proteome, and metabolome of single cells can provide valuable information on the biochemical function and phenotype of cells within and across the entire population in a tissue. Spatially resolved mass spectrometry (MS) approaches, including mass spectrometry imaging (MSI), have the ability to reveal the metabolome within anatomical compartments of tissues and across populations of individual cells. Our team has developed and utilized a number of these MS techniques, including matrix-assisted laser desorption/ionization (MALDI) and laser-ablation electrospray ionization (LAESI)– amongst others— to explore a variety of plant and plant-microbe systems. Using MALDI-MSI, we were able to determine the molecular location of key metabolites and lipids within multiple plant-based interkingdom interactions. For example, our results demonstrated how metabolic asymmetry exists within specialized soybean root organs (i.e., nodules), as a function of the plant's symbiosis with soil bacteria capable of fixing nitrogen. LAESI-MS is an emerging method that has shown notable potential for spatial metabolomics of plant samples. This is in part due to LAESI-MS being an ambient ionization method that requires minimal sample preparation and uses (endogenous) water for *in situ* analysis. Recently, we reported the integration of a microscope into the optical train of our conventional LAESI source to allow for visually informed ambient *in situ* single cell analysis and MSI with improved lateral resolution. We refer to this system as the LAESI-based 'molecular microscope'. This system allows us to do targeted high-throughput single cell analysis directly from plant tissue sections, for example.

ITZEL MENESES

Universidad Nacional Autónoma de México, MX

December 13th, 2022

8:20 AM - 8:30 AM PST

Itzel Meneses is a graduate student in the Cuevas-Velazquez lab in the Biochemistry Department at Universidad Nacional Autónoma de México, where she uses IDPs to generate biosensors to track the effects of osmotic stress in plant cells.

Systematic modification of a FRET biosensor based on a intrinsically disordered protein for tracking the effects of osmotic stress in Arabidopsis

Itzel Meneses-Reyes, Cesar Cuevas-Velazquez

Departamento de Bioquímica, Facultad de Química, Universidad Nacional Autónoma de México (UNAM)

Plants are frequently exposed to abiotic stress conditions such as water deficit. Water deficit induces hyperosmotic stress conditions in cells. In response to hyperosmotic stress, plants accumulate a group of proteins known as late embryogenesis abundant (LEA) proteins. LEA proteins lack a well-defined three-dimensional structure and are considered intrinsically disordered proteins (IDPs). Recently, our laboratory exploited the disordered character of a LEA protein to develop a genetically encoded FRET biosensor that is capable of reporting the effects of osmotic stress on different organisms, including yeast, bacteria, *Nicotiana benthamiana*, and human cells. The biosensor, named Sensor Expressing Disordered protein 1 (SED1) uses the *Arabidopsis thaliana* AtLEA4-5 as the sensor domain. However, the major limitation that SED1 presents is its non-responsiveness to osmotic changes in *Arabidopsis thaliana*. Since AtLEA4-5 is an *A. thaliana* protein, the lack of response could be the result of phosphorylations that might prevent AtLEA4-5 compaction. Also, the donor (mCerulean3) fast photobleaching could directly affect the FRET efficiency. Based on these, we systematically modified certain characteristics of SED1 to generate enhanced versions that are functional in *A. thaliana*. We generated two variants: AtLEA4-5 protein incapable to be phosphorylated (SED1-phosphonull) and a construct with a different FRET pair (mTurquoise2 and mNeonGreen; SED1-mTq2-mNG). These variants were characterized in yeast cells and *N. benthamiana* leaves under hyperosmotic stress conditions. We found that the SED1-mTq2-mNG variant exhibits a FRET change comparable to the original SED1 version in yeast and plants subjected to stress. However, SED1-phosphonull exhibited a lower FRET change than SED1. Our results suggest that SED1-mTq2-mNG is a good candidate for *A. thaliana.* This study will help to obtain a functional biosensor in *A. thaliana* that allows us to dynamically track the effects of osmotic stress in living cells and will have great applications in plant cell biology.

TESSA BURCH-SMITH

Donald Danforth Plant Science Center, USA

December 13th, 2022

3:05 PM - 3:25 PM PST

Dr. Burch-Smith is a Principal Investigator at the Donald Danforth Plant Science Center. She and her lab use electron microscopy to study how plant cells communicate with each other through intercellular pores known as plasmodesmata.

Applying 3-D imaging approaches to understand how single plant cells communicate to achieve multicellularity

The development of increasingly complex multicellular forms is a hallmark of plant evolution. As such, intercellular communication is critical for proper differentiation, growth, and defense. For many eukaryotic organisms, communication can be achieved via direct membrane contacts, but for plants enveloped in cellulosic cell walls, direct membrane contact between adjacent cells is prevented. As a result, plants have evolved alternative mechanisms to transmit signals to neighboring cells. One of the most efficient forms of communication in plants occurs via plasmodesmata (PD). PD are small, membrane-bound pores that span the cell wall and result in cytosolic and membrane continuities between adjacent cells. Molecules ranging in size from single atoms to small RNAs and transcription factors can traffic through PD, eventually entering the vasculature for systemic communication, signaling and resource allocation between above- and belowground systems. While PD constitute a route for communication between neighboring cells and serve as the gateway to systemic signaling, many pathogens including viruses, fungi and nematodes have evolved to manipulate PD and use them for rapid spread throughout the plant. Here, we demonstrate that advanced imaging techniques can be adapted and adopted for use in plants. We have used investigations of the 3D ultrastructure of PD and the distribution of these pores at various cell-cell interfaces to illuminate the value of 3-D imaging approaches such as dual-axis transmission electron microscopy tomography, focused ion beam and serial block-face scanning electron microscopy. Generating a comprehensive 3-D structural catalog of plant cells, tissues and organs will critically augment the power and context of emerging single-cell datasets. Furthermore, combining these modalities will yield a more comprehensive, informative and effective atlas, laying the foundation for probing longstanding questions in plant development and survival that are routed in plant multicellularity.

NOÉ PERRON

University of Florida, USA

December 13th, 2022

3:35 PM - 3:45 PM PST

Noé Perron is a Ph.D. student in the Department of Plant Molecular and Cellular Biology at University of Florida, working in the Kirst lab on photosynthetic adaptations to drought.

The ice plant genome and single-cell transcriptome offer new insights into the expression changes during the C3 to CAM transition

Noé Perron (1), Christopher Dervinis (2), Brad Barbazuk (3), Sixue Chen (4), and Matias Kirst (1,2)

1: Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL, USA
2: School of Forest, Fisheries and Geomatics Sciences, University of Florida, Gainesville, FL, USA
3: Department of Biology, University of Florida, Gainesville, FL, USA
4: Department of Biology, University of Mississippi, Oxford, MS, USA

A considerable challenge for humanity will be to adapt agricultural production to feed a rapidly growing population and to increase biofuel production in a changing climate. With the frequency of drought events increasing at an alarming rate and water resources becoming scarcer, finding ways to improve plant water use efficiency and resilience to abiotic stress is a priority. While most plants on earth use the C3 or C4 pathways as their photosynthetic mechanism, some plants have evolved the Crassulacean acid metabolism (CAM) to adapt to arid conditions. Research on CAM has recently attracted great interest in the plant science community because the water-conserving characteristics associated with this pathway could be transferred to crops to make them drought resistant. However, the general lack of understanding of this pathway and the limited genomic information available for some of the most studied CAM-performing species has hampered research in this area.

Here we present the genome and single-cell transcriptome of *Mesembryanthemum crystallinum*, the common ice plant. The ice plant is a facultative CAM – it grows normally using only C3 but can reversibly induce CAM when subjected to high salinity or drought. Thus, it represents an excellent system for studying the CAM induction process. The 367.91 Mb long genome was assembled using long-reads generated by the ONT PromethION platform and short Illumina reads. In total, 97.8% of the BUSCO genes were found using the embryophyta set, indicating high genome completeness, and the assembled sequence has a contig N50 of 7.19 Mb. In addition, the transcriptomes of ice plant roots, stems, and leaves were sequenced using the PacBio Iso-Seq method to support annotation. Finally, we present the first findings of our single-cell transcriptome study of the C3 to CAM transition in the ice plant. Our data suggest that distinct cell types respond differently to the application of salt stress, with highly heterogeneous expression of CAM-related genes between cell types after 8 days of treatment. Furthermore, our results identified clear cell type-specific shifts in the circadian expression patterns of key CAM genes in salt-stressed plants.

CHIN-MIN KIMMY HO

Academia Sinica, Taiwan, R.O.C.

December 13th, 2022

3:50 PM - 4:10 PM PST

Dr. Chin-Min Ho is an Assistant Research Fellow at the Institute of Plant and Microbial Biology (IPMB) at Academia Sinica. She studies factors in leaf epidermal development, which could help unlock how to generate a climate impact-resistant epidermis.

Leaf epidermal patterning and fate determination

Chin-Min Kimmy Ho

Institute of Plant and Microbial Biology, Academia Sinica

As the interface between plants and the environment, the leaf epidermis provides the first layer of protection against dehydration, UV and biotic stresses. Stomata, microscopic pores in the epidermis, function as valves to control gas exchange for photosynthesis as well as water transpiration between plants and the atmosphere. Recent studies have demonstrated that decreasing the number of stomata in rice epidermis or increasing the dynamics of stomatal movement enhances the drought tolerance and yields. While the jigsaw-shaped pavement cells serve as building blocks for leaf integrity, the cuticle layers on top of the pavement cells together with stomata balance the trade-off between photosynthesis and water loss. About sixty percent of Arabidopsis leaf epidermal cells are made through stomatal development. An asymmetric cell division initiates the lineage by producing a small stomatal lineage precursor cell, and a large Stomatal Lineage Ground Cell (SLGC). Interestingly, SLGCs could either go differentiation or re-enter asymmetric cell division to produce more stomatal linage cells. What “flips the switch” drives a cell to exit from a proliferative state to a differentiation state is not known. Using cell-type-specific markers combined with FACS, we were able to identify the transcriptome signatures of SLGCs. Further analysis of the candidate genes, we found the biochemical signaling and mechanical properties are integrated to form a functional tissue. In addition, we found chromatin organization influences a cell state switch from proliferation to differentiation, thereby, rewiring leaf epidermal patterning.

CAROLINE MARTIN

Massachusetts Institute of Technology, USA

December 13th, 2022

4:20 PM - 4:30 PM PST

Carly Martin is a Ph.D. student at the Massachusetts Institute of Technology in the Gehring lab Whitehead Institute. She is currently using seed single-nuclei sequencing to map signatures of selection from genes to specific cell types in Arabidopsis.

Dissecting seed tissue coordination through development with snRNA-sequencing

Carly Martin, Mary Gehring

MIT Biology and the Whitehead Institute for Biomedical Research

Successful angiosperm seed development requires the coordination of seed coat, endosperm, and embryonic tissues. The molecular crosstalk that occurs between these tissues is only beginning to be understood and may elucidate important targets for seed engineering. The nature of inter-tissue communication and resource partitioning in seeds is likely shaped by the unique relatedness pattern of the three principal seed tissues and the parental genetic conflicts therein. The seed coat is a fully maternal tissue while the diploid embryo and triploid endosperm, which develop upon fertilization, are genetically identical except for the additional maternal genome complement in the endosperm. The endosperm plays an important nutritive role for the embryo and is proposed to be a site of genetic parental conflict, which arises when paternal and maternal resource investment into offspring is asymmetric. Parental conflict in the endosperm drove the evolution of imprinting in this tissue, but how parental conflict shaped the evolution of genes and pathways involved in developmental signaling and resource allocation processes in the seed is not yet appreciated. We are using whole seed single-nuclei RNA-sequencing and evolutionary genomics to map signatures of selection at single-nuclei resolution through seed development. In our atlas of ~47,000 nuclei from day after pollination (DAP) 5 and DAP7, we have identified genes under positive, purifying, and neutral selection in all seed tissues. We find that endosperm subtypes closest to the maternal-filial boundary are enriched for genes under positive selection. We hypothesize that this signature of selection may be a consequence of parental conflict and that the genes exhibiting the greatest positive selection in this region are critical for proper resource partitioning in the seed any may represent an adversarial axis of the filial-maternal relationship.

Poster 01

KAMAL KUMAR MALUKANI

Tata Institute for Genetics and Society, Bangalore, India

Large scale rice transcriptome analysis reveals unique pathways and defense genes involved in rice-Xanthomonas interaction

Kamal Kumar Malukani (1,2), Gokulan C.G (1), Namami Gaur (1,3), Hitendra K. Patel (1), Ramesh V. Sonti (1,4)

1: CSIR-Centre for Cellular and Molecular Biology, India
2: Tata Institute for Genetics and Society, India
3: University of Dundee, U.K.
4: Indian Institute of Science Education and Research-Tirupati, India

Xanthomonas oryzae is a severe pathogen of rice that causes bacterial blight and bacterial leaf streak diseases. Different laboratories have performed transcriptomics analysis in various conditions to understand this complex rice-Xanthomonas interaction. This includes compatible interactions, incompatible interactions, and treatment with pathogen-secreted cell wall degrading enzymes, which induces rice immune responses. We retrieved this data available in Gene expression omnibus (NCBI-GEO) and analyzed it. We generated a database, which will be released soon. We categorized collected datasets in compatible and incompatible interactions and further classified those based on sample collection time post-Xanthomonas treatment.

Pathway analysis of very common differentially expressed genes indicates significant enrichment of genes involved in many pathways not much explored in plant-pathogen interaction. These pathways include plant global translation, ribosome biogenesis, pseudouridine synthesis, RNA editing, circadian rhythm, membrane trafficking, vitamin synthesis, and growth-related hormone ABA. We also observed pathways altered at early (before 24hr) time points following Xanthomonas treatment are very common between compatible and incompatible interaction, and these pathways also overlap with altered pathways following CWDE treatment. We also narrowed down to list of genes very commonly differentially expressed following Xanthomonas treatment. To add to our confidence, some of these have already been reported as defense-related genes, while the other genes appear suitable candidates for further study. We transiently overexpressed three of these putative defense related genes and observed their overexpression leads to enhanced expression of key rice defense-related marker genes and provides tolerance against subsequent bacterial blight infection. Overall, our study indicates some unexplored rice pathways are altered following treatment of Xanthomonas. We also propose some new rice genes, which can be further explored to understand their role in plant-microbe interaction.

Poster 02

ZHENGYAO SHAO

The University of Texas at Austin, USA

Phosphorylation Status of B beta Subunit Acts as a Switch to Regulate the Function of Phosphatase PP2A in Ethylene-mediated Root Growth Inhibition

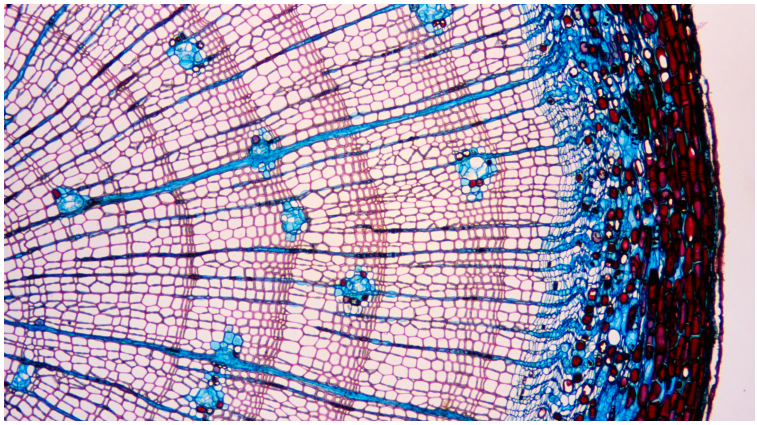
Zhengyao Shao (1,2), Bo Zhao (1,2), Prashanth Kotla (1), Jackson G. Burns (1), Jaclyn Tran (1,2), Meiyu Ke (3), Xu Chen (3), Karen S. Browning (1,2) and Hong Qiao (1,2)

1: Institute for Cellular and Molecular Biology, The University of Texas at Austin, USA
2: Department of Molecular Biosciences, The University of Texas at Austin, USA
3: Haixia Institute of Science and Technology, Horticultural Plant Biology and Metabolomics Center, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China

The various combinations and regulations of different subunits of phosphatase PP2A holoenzymes underlie their functional complexity and importance. We found that phosphorylation status of Bβ of PP2A acts as a switch to regulate the activity of PP2A. In the absence of ethylene, phosphorylated Bβ leads to an inactivation of PP2A; the substrate EIR1 remains to be phosphorylated, preventing the EIR1 mediated auxin transport in epidermis, leading to normal root growth. Upon the ethylene treatment, the dephosphorylated Bβ mediates the formation of A2-C4-Bβ protein complex to activate PP2A, resulting in the dephosphorylation of EIR1 to promote auxin transport in epidermis of elongation zone, leading to root growth inhibition. Altogether, our research revealed a novel molecular mechanism by which the dephosphorylation of Bβ subunit switches on the PP2A activity to dephosphorylate EIR1 to establish EIR1 mediated auxin transport in epidermis in elongation zone for root growth inhibition in response to ethylene.

Poster 03

JENNIFER
LÓPEZ ORTIZ
University of Helsinki, Finland



Separation of cork and vascular cambia identities from a single cell

Jennifer López Ortiz (1,2), Hiroyuki Iida (1,2), Ari Pekka Mähönen (1,2)

1: Institute of Biotechnology, HiLIFE, University of Helsinki, Finland
2: Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, and Viikki Plant Science Centre, University of Helsinki, Finland

When organs undergo secondary growth, cork and vascular cambia are established as lateral meristems in most dicots. The vascular cambium is nested inside the cork cambium, and these cambia provide thickness to stems and other organs. The vascular cambium is the inner lateral meristem and produces the conductive tissues –secondary xylem and phloem. The cork cambium provides the protective barrier, the cork layer, against abiotic and biotic stresses in organs undergoing secondary growth. In Arabidopsis roots, the vascular cambium emerges from procambial cells; the cork cambium arises from pericycle cells. Recently, from lineage tracing analysis, we have discovered that the xylem pole pericycle (XPP) cell contributes to the formation of both vascular and cork cambia. The XPP cell makes it possible to form a radially symmetric pattern in secondary tissue from a bisymmetric pattern in the primary root vasculature. When the XPP cells fail to produce either cambium, secondary tissue formation is severely disturbed and plant fitness is diminished. Therefore, the contribution of XPP cell lineage to both cambia is critical for the proper patterning. However, little is known about how cork and vascular cambia identities are diverged in the XPP cell lineage. By reporter analysis, we found that this identity separation happens in the early stage of secondary growth initiation. In this presentation, I will present how these identities are diverged in the XPP cell lineage and discuss potential mechanisms underlying the separation processes.

Poster 04

YASIR ARAFAT TAMAL

Max Planck Institute for Plant Breeding Research, Germany

Non-negative matrix factorization (NMF) is applied to infer cellular composition and constituent gene expression programs (GEPs) from scRNAseq data

Yasir Arafat Tamal, Stefan Laurent

Department of Comparative Development and Genetics, Max Planck Institute for Plant Breeding Research, Cologne, Germany

The tissue sample of interest in developmental studies is frequently a heterogeneous mixture of several subpopulations of cells in different developmental states. It is necessary to generate functional genomic data at single-cell resolution in order to identify the constituent cell types and the gene expression programs (GEPs) that characterize these cell types. Single-cell RNA sequencing has enabled researchers to generate gene expression profiles for thousands of cells, while also providing high-resolution data on cellular phenotypes and developmental dynamics.

Cellular gene expression profiles are the result of a complex interplay of multiple related biological programs, where the expression profile may reflect a mixture of the cell's core identity program and other developmental time-specific cellular activity programs such as the cell cycle. Genes are induced together as a GEP through complex transcriptional co-regulation to establish a cell's identity when it differentiates from multipotent progenitors to a more differentiated state, to maintain the identity of the developmental state, or to respond to external stimuli and environmental cues, or to carry out complex cellular activities. Since individual cells can express activity expression programs in addition to the core identity expression program, these GEPs can be divided into two types: identity GEP and activity GEP. Identity GEPs define a specific cell type, such as the epidermis cell type, whereas activity GEPs are expressed independently in a cell where a specific cellular activity, such as cell cycle, is taking place. Because of the intrinsic ability of scRNA-seq to capture variation in gene expression across many individual cells, it offers us the requisite potential to detect GEPs from quantified transcriptome profiles. But due to the substantial drop-out effect and noisy nature of the high-dimensional scRNA-seq data, inferring the GEPs becomes difficult, necessitating analytical approaches to detect the underlying structure. In this project, I used a matrix factorization technique called non-negative matrix factorization (NMF) to infer the molecular makeup of cells by identifying identity GEPs and activity GEPs from transcriptome data and using this information to estimate the cellular makeup of *Cardamine hirsuta* and *Arabidopsis thaliana*. Despite extensive studies on the model plant *A. thaliana*, a comprehensive analysis of the identity of every cell in the early primordia of *C. hirsuta* is lacking. REDUCED COMPLEXITY (RCO) is a key developmental gene present in *C. hirsuta*, but not in *A. thaliana*, responsible for complex leaf phenotype in *C. hirsuta* compared to simple leaf phenotype in *A. thaliana*. The primary goals of this study are to define the RCO cell type that can describe the complex phenotype of *C. hirsuta*, to establish cellular taxonomy, and to identify signature identity GEPs. Another intriguing goal is to determine the transcriptional dynamics of cellular differentiation. Integrative analysis of *C. hirsuta* and *A. thaliana* further reveals conserved and distinct features between these two species.

Poster 05

ROGELIO RODRÍGUEZ-SOTRES

Universidad Nacional Autónoma de México, México

Comparative *in silico* analysis of the Haloacid Dehalogenase PS2-like enzymes in *Capsicum annum* and *Arabidopsis thaliana*

Rogelio Rodríguez-Sotres, Lilian Valencia-Turcotte, Evelyn Bellmunt-Espíndola, Juan Carlos Islas Arciga and Alfonso Zamora-Hernandez

Departamento de Bioquímica, Facultad de Química, Universidad Nacional Autónoma de México.

The Haloacid Dehalogenase superfamily (HAD) groups very diverse proteins mostly, though not exclusively, having phophohydrolase enzymatic activity. HAD members are distributed in all three domains of life and have been related to a wide range of physiological functions in yeast, bacteria and animals. While these enzymes have received limited attention in plants, one branch includes the phosphate-starvation 2 protein (PS2), which is highly expressed under phosphate deprivation conditions in all plant species where the response has been studied. This protein was originally considered as a phosphatase, but it was later found to have pyrophosphatase activity. A closely related relative of this protein has been found to have thiamine phosphate phosphatase activity.

There are three PS2 close paralogs in *A. thaliana,* and in *C. annuum* related sequences were found, though the annotation state of the chilly pepper genome is still rather imperfect, and it is not clear that all sequences are expressed and become active enzymes.

Taking advantage of Alpha Fold 2.0 structural predictions for the entire UniprotKB, a comparison of the putative active sites of the members of this family was done for both plants. We found a putative Cys-4 Zn finger connected to the active site in all these predicted structures, which seems a distinctive characteristic of this subgroup of the HAD superfamily. This Zinc site may modify the electronic properties of a putative catalytic lysine, highly conserved in the entire HAD superfamily.

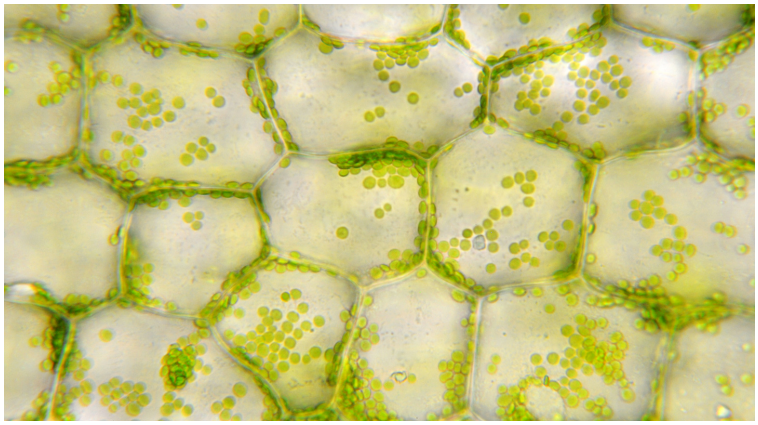
To probe *in silico* these enzymes' specificity, and find evidence for a role of Zinc in substrate binding, we used Molecular Docking and QM PM7 semiempirical calculations for two members of this group: one from Arabidopssis, and one from chili pepper. Using the localized molecular orbitals (LMO) approach, it was possible to obtain a QM consistent geometry and approximate the electronic structure for these two entire proteins, with implicit COSMO solvation.

From the results, these enzymes recognize 4-phosphonoxy-lysine with the strongest binding energy, while phosphoethanolamine also showed significant binding enthalpy, but the recognition of pyrophosphate was considerably weaker. Our results point to a role for this enzyme in amino acid metabolism, perhaps phospholipid metabolism, and question the proposal of this enzyme acting a pyrophosphatase *in vivo*. Our data still need experimental confirmation, but are more in line with the finding of a role for these enzymes in phospholipid recycling.

Poster 06

ROSALIE SINCLAIR

University of California, Davis, USA



Spatiotemporal Dynamics Of Cell Plate Development During Plant Cytokinesis

Rosalie Sinclair (1), Thomas Wilkop (2), Zaki Jawaid (3), Jesse Aaron (4), Blair Rossetti (4), Eric Wait (4), John Heddleston (4), Daniel Cox (3), Georgia Drakakaki (1)

1: Department of Plant Sciences University of California, Davis, USA
2: Department of Molecular Cellular Biology University of California, Davis Light Microscope Core, USA
3: Department of Physics University of California, Davis, USA
4: Janelia Research Campus, Ashburn, USA

Cytokinesis in plants is fundamentally different from that in animals and fungi. In plant cells, a cell plate forms through the fusion of cytokinetic vesicles and then develops into the new cell wall, partitioning the cytoplasm of the dividing cell. The formation of the cell plate entails multiple stages that involve highly orchestrated vesicle accumulation, fusion and membrane maturation, which occur concurrently with the timely deposition of polysaccharides such as callose, cellulose and cross-linking glycans. The polysaccharide callose is thought to play a vital role in stabilizing the cell plate and contributing to cell plate maturation. However, despite the central role of cell plate formation, many fundamental questions remain. To overcome hurdles caused by genetic lethality, we use pharmacological inhibitors, such as endosidin 7 (ES7) that specifically inhibits callose deposition during cytokinesis. Using 4D imaging, the temporal dynamics at the cell plate with and without the presence of callose have been interrogated. Imaging complete cell plate events can identify key transition points at which different components such as callose are critical for cell plate development. Quantitative analysis, along with biophysical modeling, can help us dissect the molecular black box surrounding callose deposition during cytokinesis and endomembrane dynamics at the cell plate.

Poster 07

XIAOSA XU

Cold Spring Harbor Laboratory, USA

Single-cell analysis of plant shoot meristems opens a 'goldmine' for functional studies

Xiaosa Xu (1), Michael Passalacqua (1), Jesse Gillis (1,2), David Jackson (1)

1: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA
2: University of Toronto, Toronto, Ontario, Canada

Plant shoot meristems determine plant architecture and impact crop productivity. An understanding of plant shoot meristems requires insights into developmental domains, and the gene networks required to specify them. However, these domains are classified mainly by morphology, or insights from classical genetics, but this knowledge is limited by genetic redundancy and pleiotropy.

Here, we firstly constructed a single-cell gene expression atlas of maize inflorescence shoot meristems. We revealed novel developmental markers and validated them by bulk RNA-seq and mRNA *in situ*. Next, we created the gene co-expression network at single-cell resolution to predict genetic redundancy. We also integrated transcription factors ChIP-Seq with single-cell data to build transcriptional regulatory networks. Finally, we combined Genome-Wide Association Studies with single-cell data to identify yield-associated genes.

We further successfully captured rare stem cells in Arabidopsis and maize shoot meristems that were largely missed in previous plant shoot single-cell studies. We identified stem cell markers and validated their expression using a high-resolution spatial analysis approach. We conducted a cross-species single-cell analysis to discover conserved stem cell markers and cell types. We also constructed the developmental trajectories from undifferentiated stem cells to differentiated cells.

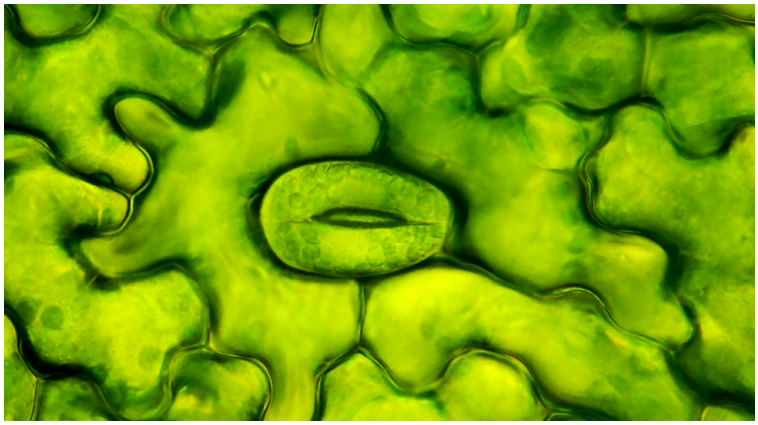
Plant stem cells are maintained by a conserved CLAVATA-WUSCHEL (CLV-WUS) pathway. We thus also profiled single cells from the inflorescence shoot apices of mutants of crucial regulators in CLV-WUS pathway. We found hundreds of differential expressed genes (DEGs) in the stem cells by comparing these mutants with wild type. We used multiplex CRISPR/Cas9 to knock out selected DEGs in a family of sugar kinases, and found a striking meristem termination phenotype, validating the predictive power of our single-cell atlas.

Together, this comprehensive plant shoot meristem single-cell atlas opened a 'goldmine' for functional studies at a fundamentally new level, which will be a valuable resource for the plant community.

Poster 08

LE LIU

University of Massachusetts, USA



A Receptor-Like Protein PAN2 is required for ABA and dark-mediated grass stomatal closure via its expression on the subsidiary cell plasma membrane

Le Liu(1), M. Arif Ashraf, Taylor Morrow (1,⁎), Michelle Facette (1)

1: Plant Biology Graduate Program and Department of Biology, University of Massachusetts, Amherst, USA
⁎Undergraduate Researcher

Stomata are small pores on the leaf surface of land plants that facilitate gas exchange. Grass stomata have fast stomatal responses, which may be associated with the dumbbell-shaped guard cells and lateral subsidiary cells. Subsidiary cells are thought to exchange water and ions with guard cells reciprocally. However, the relative contribution of subsidiary cells and the mechanisms of how they contribute are unclear. To untangle the role of subsidiary cells in stomatal closure, we measured stomatal function in *pan1*, *pan2*, and *pan1pan2*, mutants. PAN2 and PAN1 are receptor-like proteins essential for correct subsidiary cell formation. In *pan1* or *pan2* single mutants, ~25% of the stomata are abnormal in juvenile leaves, but <5% are abnormal in adult leaves. We measured gas exchange in juvenile and mature leaves of *pan1*, *pan2*, and *pan1pan2* mutants to determine if correct subsidiary cell formation was required for stomatal function. Both juvenile and adult leaves from *pan2* and *pan2*; *pan1* show slower stomatal closing dynamics in the dark relative to controls. However, even though *pan1* and *pan2* have similar numbers of defective subsidiary cells, the gas exchange dynamics of *pan1-ems* were identical to B73. This suggests that PAN2 has a role in stomatal function independent of subsidiary cell morphology. To determine if the stomatal function of individual stomatal complexes is dependent on correct subsidiary cell specification, we measured stomatal aperture in different genotypes of plants treated with ABA. In all genotypes, stomata with abnormal subsidiary cells show closure defects in response to ABA. **This is direct evidence that correct subsidiary cells are essential for closing the stomatal pore.** Together, our results indicate that PAN2 is critical for stomatal function and regulation of stomatal aperture, even in normally formed stomata of mature leaves. PAN1 is important for stomatal complex formation but does not seem as crucial for the function of normal stomata.

Poster 9

ANG-YU LIU

Iowa State University, USA

The purple acid phosphatase PAP26 is essential for efficient nucleotide metabolism in the vacuole of Arabidopsis

Ang-Yu Liu (1,2), and Gustavo MacIntosh (1,2)

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RNA is one of the major macromolecules found in all forms of life, yet bulk RNA turnover in plants has only been reported recently, and our knowledge of the vacuolar salvage pathway is incomplete. RNS2, a vacuolar ribonuclease, catalyzes ribosomal RNA (rRNA) degradation in Arabidopsis. The *rns2-2* mutant displays a longer half-life of rRNA along with bulk RNA accumulation in the vacuole, and this mutant also shows an upregulation of basal autophagy as a consequence of low TOR activity. As this phenotype can be rescued by treating the mutant plant with inosine, a purine nucleoside precursor, vacuolar RNA degradation is proposed to be an important contributor to maintain the level of cytosolic nucleotides.

However, how nucleosides are generated from RNS2-dependent RNA degradation remains unclear. We characterized the pathway of nucleotide metabolism and its physiological implications using a combination of *in vitro* assays and molecular genetic approaches. The *in-vitro* degradation of polyadenylic acid showed that the presence of PAP26, a purple acid phosphatase responsible for 80 % of the acid phosphatase activity in the vacuole, changes the ratio of adenosine and 2′ 3′ cyclic adenosine monophosphate (2′ 3′ cAMP) produced by purified vacuoles. Further investigation showed that purified vacuoles produce 3′ adenosine monophosphate (3′ AMP) from catalyzing the hydrolysis of 2′ 3′ cAMP, but the conversion of 3′ AMP into adenosine is reduced in the reaction catalyzed by vacuole extracts of the *pap26* mutant. In addition, the analyses of vacuolar metabolites revealed that loss of PAP26 activity changes the relative levels of nucleotide metabolites including 2′ 3′ cyclic uridine monophosphate (2′ 3′ cUMP), 3′ uridine monophosphate (3′ UMP), and uridine, revealing that PAP26 catalyzes nucleotide turnover in the vacuole. The *pap26* mutant also displays increased basal autophagy that can be rescued by inosine treatment, indicating that the involvement of PAP26 in nucleotide metabolism is critical to the nucleoside salvage pathway of Arabidopsis. We propose that PAP26 could also have a role in regulating RNS2 kinetics by changing the chemical equilibrium through products removal, which would increase the catalytic efficiency of RNA degradation in the vacuole.

Poster 10

ANSAR ALI

Institute of Plant and Microbial Biology,
Academia Sinica

HOMEODOMAIN containing protein mediates chromatin compaction and rewires leaf epidermal patterning

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The chromatin landscape must coordinate cell proliferation and differentiation to sustain appropriate development and tissue patterning. The process of proliferation and differentiation in developing leaf epidermis is not entirely understood. Here we have identified a homeodomain-like superfamily protein (HDL) in Arabidopsis. HDL is found in chromocenters and is necessary for heterochromatin organization. The loss of HDL reduced the number of heterochromatin foci and the de-repression of transposons elements. We found that expression of stomatal-related genes is greatly reduced in the *hdl* mutant, which is consistent with *hdl* phenotypes with a lower stomatal density (diploid) and an increased trichomes density (polyploid). ChIP-seq study of HDL revealed enhanced binds in histone deacetylation and modification genes. Additional research revealed that HDL interacts with histone deacetylase 6 (HDA6) and methyltransferase (KYP), implying a role in chromatin compaction. We discovered that *hdl* plants have more accessible chromatin regions than WT plants in both euchromatin and heterochromatin areas using an accessibility assay. Furthermore, our findings show that *hdl* progresses slowly through the G1 phase. Our results indicate that chromatin compaction increased the likelihood of developmental choices, perhaps delaying the transition from proliferation to differentiation status via altering the cell cycle.

Poster 11

MANUEL NEUMANN

Humboldt University of Berlin, Germany

Spatial reconstruction of single-cell gene expression in floral meristems of *Arabidopsis thaliana*

Manuel Neumann, Xiaocai Xu, Kerstin Kaufmann, Jose M Muino

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The relative position of cells in a plant structure is important to determine their developmental trajectories and thus the changing morphology of developing plant structures. For that reason, to understand developmental processes in plants, it is important to link transcriptomic dynamics at single-cell resolution with spatial information during plant development.

Combining single-cell -omics technologies with confocal imaging provides great opportunities to chart cellular identities along development in terms of gene expression and chromatin status. The spatial information of cells is lost in conventional single-cell -omics experiments, as a result of tissue dissociation during sample preparation. New spatial transcriptomics technologies allow one to obtain spatial information of gene expression. But the resolution or number of profiled genes is limited.

Here, we show how to overcome those limitations by predicting spatial gene expression profiles in the *Arabidopsis thaliana* floral meristem by computationally integrating 3D confocal microscopy-based expression of ~25 marker genes with scRNA-seq data of flower meristems. This allowed us to visualize and understand differences in spatial gene expression profiles among different meristem domains.

Together, our work demonstrates how spatially reconstructed scRNA-seq data can be used to shed light on the emerging cellular heterogeneity in developing meristems at single-cell and spatial resolution.

Poster 12

ARIF ASHRAF

University of Massachusetts Amherst, USA

The nucleus decides the future division site

M. Arif Ashraf and Michelle Facette

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Both plants and animals rely on asymmetric cell division to generate new cell types and is a core characteristic of multicellular organisms. Prior to asymmetric cell division, cell polarity is established. Cell polarity establishment and asymmetric cell division are universally important, although proteins important for polarity differ in plants and animals. *Zea mays* stomatal development serves as an excellent plant model system for asymmetric cell division, especially the asymmetric division of the subsidiary mother cell (SMC). In SMCs, the nucleus migrates to a polar location, after the accumulation of polarly localized proteins but before the appearance of the preprophase band. We examined a mutant of the outer nuclear membrane protein, which is part of the LINC (linker of nucleoskeleton and cytoskeleton) complex that localizes to the nuclear envelope in interphase cells. Previously, *mlks2* (*maize linc kash sine-like2*) were observed to have abnormal stomata. We confirmed these stomatal defects and used cell markers to pinpoint the precise defects that lead to abnormal asymmetric divisions. Proteins that are polarly localized in SMCs prior to division, including a polar accumulation of actin, polarize normally in *mlks2* and remain polarized throughout and after division. However, polar localization of the nucleus is impaired, even in cells that have otherwise normal polarity. This leads to a misaligned preprophase band and atypical division planes Localization of MLKS2 revealed it is able to associate with mitotic structures, however the structure of the preprophase band, spindle and phragmoplast appeared normal in *mlks2*. Time-lapse imaging in *mlks2* revealed that the mutant had defects in pre-mitotic nuclear migration towards the polarized site, as well as an unstable position at the division site after formation of the preprophase band. Unpolarized nuclei lead to a misaligned preprophase band. Together, we show compelling genetic evidence that nuclear envelope proteins promote pre-mitotic nuclear migration and stable nuclear position, and that the position of the nucleus influences division plane establishment in asymmetrically dividing cells.

Poster 13

FENG ZHAO

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Northwestern Polytechnical University,
China

Auxin guides germ-cell specification in Arabidopsis anthers

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2: College of Life Sciences, Peking University, Beijing, China
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Germ cells (GCs) are the key carriers delivering genetic information from one generation to the next. In a majority of animals, GCs segregate from somatic cells during embryogenesis by forming germlines. In land plants, GCs segregate from somatic cells during postembryonic development. In a majority of angiosperms, male GCs (archesporial cells) initiate at the four corners of the anther primordia. Little is known about the mechanism underlying this initiation. Here, we discovered that the dynamic auxin distribution in developing anthers coincided with GC initiation. A centripetal auxin gradient gradually formed toward the four corners where GCs will initiate. Local auxin biosynthesis was necessary for this patterning and for GC specification. The GC determinant protein SPOROCYTELESS/NOZZLE (SPL/NZZ) mediated the effect of auxin on GC specification and modified auxin biosynthesis to maintain a centripetal auxin distribution. Our work reveals that auxin is a key factor guiding GC specification in Arabidopsis anthers. Moreover, we demonstrate that the GC segregation from somatic cells is not a simple switch on/off event but rather a complicated process that involves a dynamic feedback circuit among local auxin biosynthesis, transcription of SPL/NZZ, and a progressive GC specification. This finding sheds light on the mystery of how zygote-derived somatic cells diverge into GCs in plants.

Poster 14

SHIRSA PALIT

Indian Institute of Science Education and Research (IISER-Pune), Pune, India

Secretory peptides of the bryophyte-specific protein SHORT-LEAF regulate gametophore development in moss *P. patens*

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Land plants have evolved elaborate developmental programs intricately interconnected by genes and phytohormones. Bryophytes such as mosses share their common ancestor with angiosperms and possess functionally conserved phytohormone signaling pathways. However, reverse genetics studies of conserved flowering plant genes in moss show very limited functional conservation, emphasizing the need to study the role of clade-specific proteins in phytohormone mediated plant development. We have recently reported isolation and characterization of the *short-leaf (shlf)* mutant from retrotransposon based forward genetic screen. *shlf* has a characteristic short-leaf phenotype and differential auxin distribution pattern caused by a Tnt1 insertion in the SHORT LEAF gene, encoding a unique bryophyte-specific protein SHORT-LEAF (SHLF). However, the molecular role of SHLF in moss gametophore development was elusive, due to lack of any known conserved domains.

SHLF encodes a N-terminal signal peptide (N), four highly similar Tandem Direct Repeats (TDR 1-4) and a C-terminal tail (C). Our recent efforts to validate the secretory role of signal peptide, via mass spectrometric analysis of wild type (WT) secretome resulted in the identification of several SHLF peptides. Overexpression of a construct lacking signal peptide failed to recover the *shlf* mutant phenotype and SHLF peptides were absent in the secretome, indicating that secretion of SHLF is a prerequisite for protein function. Supplementation of both WT peptidome and secretome to *shlf* mutant exhibited partial and full phenotype recovery respectively, without any change in the SHLF transcript levels. In a quest to find a minimal functional SHLF, we also discovered the importance of the 2nd TDR in SHLF function and identified several secretory cryptic peptides (cryptides) derived from it. Supplementation of 2nd TDR specific synthetic peptides rescued the *shlf* phenotypes, including leaf dimensions, auxin distribution patterns, internodal distance and apical dominance. These data suggest that SHLF peptides may function in-tandem with auxin to regulate moss gametophore development. Further, transcriptomic analysis and metabolomic profiling of *shlf* showed the differential regulation of several auxin regulated genes and metabolites, which were reverted to their basal levels upon SHLF complementation.

In future, we plan to study the interacting partners of SHLF and to also elucidate the role of SHLF peptides in flowering plants, which will shed light on the evolutionary conservation of peptide signaling in plant development.

Poster 15

DIANA RUGGIERO

Oregon State University, USA

Single-cell genomics and high-throughput phenotyping for determining the quantitative genetics of maize leaf vascular development

Maize tissues that engage in efficient C4 photosynthesis have high vein density. Vascular density varies across the sheath, auricle, and blade compartments of the leaf. Although mature leaf veins are anatomically similar, maize and other C4 grasses have several different vein types occurring in a stereotypical developmental sequence and spatial configuration throughout the sheath, auricle and blade.

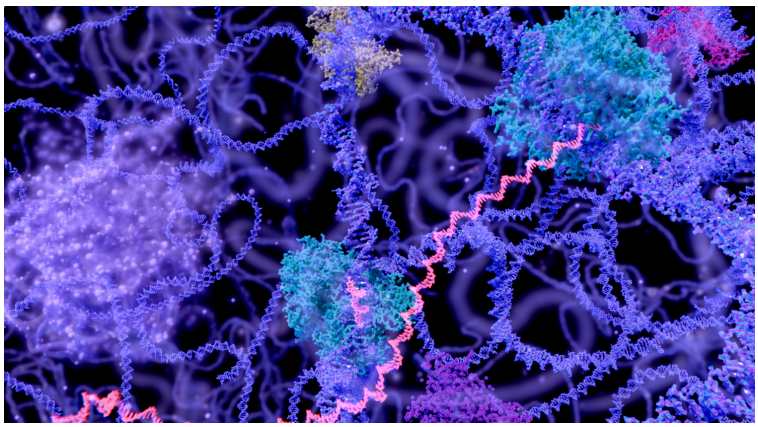
To characterize the gene expression and regulatory environments that distinguish these diverse vascular cell types within the maize leaf primordia, we used combinatorial barcoding to perform single nuclei RNA sequencing (snRNA-seq). With the Parse Single Cell Whole Transcriptome Kit, we obtained transcriptomic profiles for ~6000 individual nuclei from primordial tissue at different stages of development. We intend to use high-resolution transcriptomic data to isolate the expression patterns of different types of maize vascular tissue and characterize the genetic signals and developmental trajectories of intermediate cell types transitioning into vascular tissue.

To screen for natural variants of vascular development programs, we are conducting a GWAS correlating vein density with markers from the Wisconsin Diversity Panel (WiDiv). Over the course of two field seasons, we collected over 5000 leaf samples from more than 700 different maize lines. To facilitate this large-scale study of microscopic traits, we have devised a deep-learning based automated phenotyping system for estimating type-specific vein density in cleared leaf images. This system employs an implementation of U-Net, a convolutional neural network (CNN) architecture for semantic segmentation. This system classifies and masks the various vein types in each sample, allowing for quantification and spatial analysis of specific vascular sub-types.

Poster 16

TRAVIS LEE

Salk Institute for Biological Studies, USA



A single-nucleus transcriptome atlas of seed-to-seed development in Arabidopsis

Travis Lee (1,2,3), Tatsuya Nobori (1,2,3), Natanella Illouz-Eliaz (1,2,3), Jiaying Xu (1,2), Bruce Jow (1,2,3), Joseph Nery (2), Joseph R. Ecker (1,2,3)

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Extensive research of the model plant Arabidopsis has provided a deep understanding of organs and tissues present throughout plant development. However, we still lack a comprehensive and unified understanding of cell types and cell states across distinct tissues and organs throughout the plant life cycle at the molecular level. The demonstrated power of single-cell sequencing technologies in the identification of rare and novel cell populations motivated us to perform single-nucleus profiling along the entirety of the Arabidopsis lifecycle spanning from seed germination to embryogenesis and seed setting in mature plants. Here, we present a single-nucleus transcriptome atlas of seed-to-seed development in Arabidopsis that encompasses diverse tissues collected across ten distinct developmental stages, with an additional focus on the highly complex reproductive organs that contain many unique cell types. Analysis of over 500,000 nuclei identified over 180 major clusters and over 500 subclusters that demarcate both cell type and cell state. Cross-organ and cross-tissue analyses revealed that the transcriptional identity of many cell types is conserved across development but is also influenced by tissue of origin and developmental timing. We identified groups of transcription factors enriched and/or uniquely expressed in specific organs and developmental timepoints, suggesting a developmental gatekeeping of such transcription factors. Finally, we employed spatial transcriptomics to map 140 *de novo* identified marker genes derived from single-nucleus RNA-seq of siliques (seed capsule). These spatial gene expression data enabled us to annotate cell populations in the highly complex organ, including embryos within the context of embryogenesis inside maternal ovules.

Poster 17

IVAN RADIN

Washington University in St. Louis, USA

Evolution of vacuolar mechanosensing

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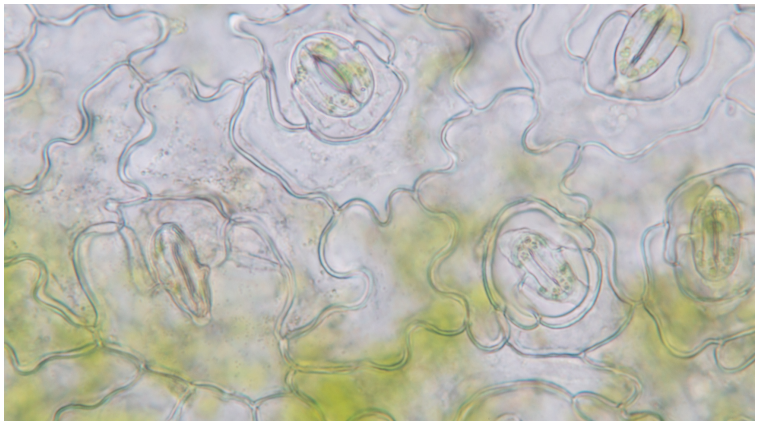
2: Department of Biological Sciences, Dartmouth College, NH, USA

The perception of mechanical forces, or mechanosensing, is an ancient and fundamental ability of all organisms. We are interested in how this process evolved within the green lineage. To facilitate cross-lineage comparisons, we worked with the flowering plant *Arabidopsis thaliana* and the moss *Physcomitrium patens*, representatives of two major land plant lineages. A simple body organization, a filamentous juvenile stage and mostly single-cell layer gametophores make moss particularly amenable to microscopic and cell biology studies. One way mechanosensing is achieved is through the action of mechanosensitive ion channels, which convert lateral membrane tension into electrochemical signals. We focused on the plant members of the PIEZO channel family, first discovered in animals where these calcium/cation channels play many essential functions including touch perception. We showed that land plant PIEZOs have dramatically diverged from their animal counterparts both with respect to their subcellular localization and their function. Unlike plasma membrane-localized animal homologs, land plant PIEZOs localize to the tonoplast and modulate vacuolar morphology in tip-growing cells such as moss caulonemata and Arabidopsis pollen tubes. The vacuoles in the tip regions of WT moss caulonemal cells have a characteristic complex tubule-like morphology, while the vacuoles in *Pp*PIEZO double mutant cells are large and expanded. Overexpression of *Pp*PIEZOs promotes tonoplast invagination into the vacuolar lumen. When we introduced gain-of-function mutations into the genomic copy of *Pp*PIEZO2, we observed dramatic vacuolar membrane invagination and novel membrane lamination. Similarly, deletion of the Arabidopsis PIEZO homolog leads to the expansion of normally filamentous vacuoles in the apical region of the tip-growing pollen tubes. We propose that land plant PIEZOs help maintain the tubule-like/filamentous vacuolar organization in tip growing cells by promoting membrane invagination and possibly fission. This vacuolar organization might facilitate the rapid growth rate of tip-growing cells. Currently, we are leveraging the fast and easy transformability of moss to ask when in evolutionary time PIEZOs were adapted to serve as putative vacuolar mechanosensors. We heterologously expressed PIEZO homologs from the Charophyte alga *Klebsormidium nitens* and the Chlorophyte alga *Chlamydomonas reinhardtii* in moss. Preliminary data suggest that *Kn*PIEZO localized to the tonoplast, while the *Cr*PIEZO was targeted to the endoplasmic reticulum. These data suggest that PIEZOs might have been adapted for the vacuolar function in the ancestor of Streptophytes (land plants and their closest algal relatives, Charophytes). We are investigating the subcellular localization of PIEZO homologs from other plant and algal species to further test this hypothesis. Altogether, it seems that in response to their unique mechanical needs, different lineages targeted PIEZOs to different cellular membranes to serve as local mechanosensors.

Poster 18

MARGOT SMIT

Stanford University, USA



Slow and not so furious: de novo stomatal pattern formation during plant embryogenesis

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Stomatal patterning needs to be both flexible and robust: adjusting to the plant's environment while maintaining spacing to ensure pore function. We characterized the de novo formation of stomatal pattern.

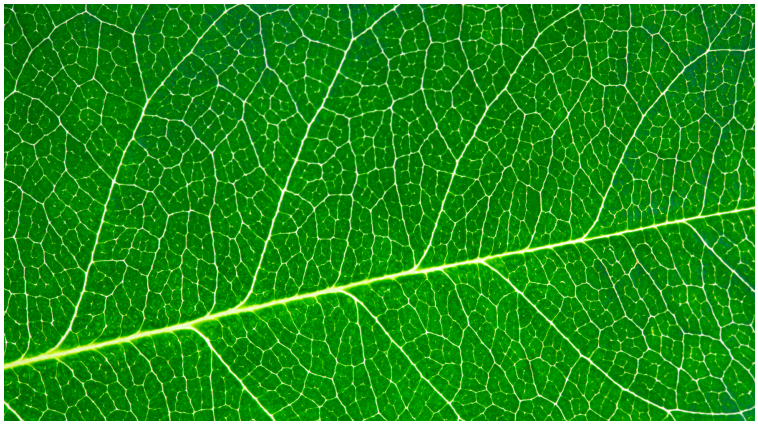
Across 35 eudicot species, stomatal patterning occurs during the last stages of embryogenesis and species that have immature embryos at seed desiccation, such as tomato, lack this pre-pattern. Considering the stomatal gene regulatory networks defined in seedlings, we found temporal uncoupling of SPEECHLESS (SPCH), which controls lineage entry, from its targets. SPCH targets display diverse patterns that may reflect organ differentiation information before condensing into expression patterns resembling those after germination. Surprisingly, ectopic SPCH expression is able to increase target expression levels but cannot induce expression earlier.

Next, the embryonic stomatal pattern arrests at GMC stage, coincident with MUTE expression. We show that when expressed during embryogenesis, MUTE and FAMA can affect gene expression, cell shape and division, but they cannot induce the formation of embryonic guard cells. Thus, the strong temporal control of stomatal development during embryogenesis cannot be circumvented through the misexpression of classical stomatal regulators.

Poster 19

PINGPING QIAN

Osaka University, Japan



A Dof-CLE circuit controls phloem organization

Pingping Qian, Tatsuo Kakimoto

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The phloem consists of sieve elements (SEs) and companion cells (CCs). Here we show that Dof-class transcription factors preferentially expressed in the phloem (phloem-Dofs) are not only necessary and sufficient for SE and CC differentiation, but also induce negative regulators of phloem development, CLAVATA3/EMBRYO SURROUNDING REGION-RELATED25 (CLE25), CLE26 and CLE45 secretory peptides. CLEs were perceived by BARELY ANY MERISTEM (BAM)-class receptors and CLAVATA3 INSENSITIVE RECEPTOR KINASE (CIK) co-receptors, and post-transcriptionally decreased phloem-Dof proteins and repressed SE and CC formation. Multiple mutations in CLE-, BAM- or CIK-class genes caused ectopic formation of SEs and CCs, producing an SE/CC cluster at each phloem region. We propose that while phloem-Dofs induce phloem cell formation, they inhibit excess phloem cell formation by inducing CLEs. Normal-positioned SE and CC precursor cells appear to overcome the effect of CLEs by reinforcing the production of phloem-Dofs through a positive feedback transcriptional regulation (Fig. 1).

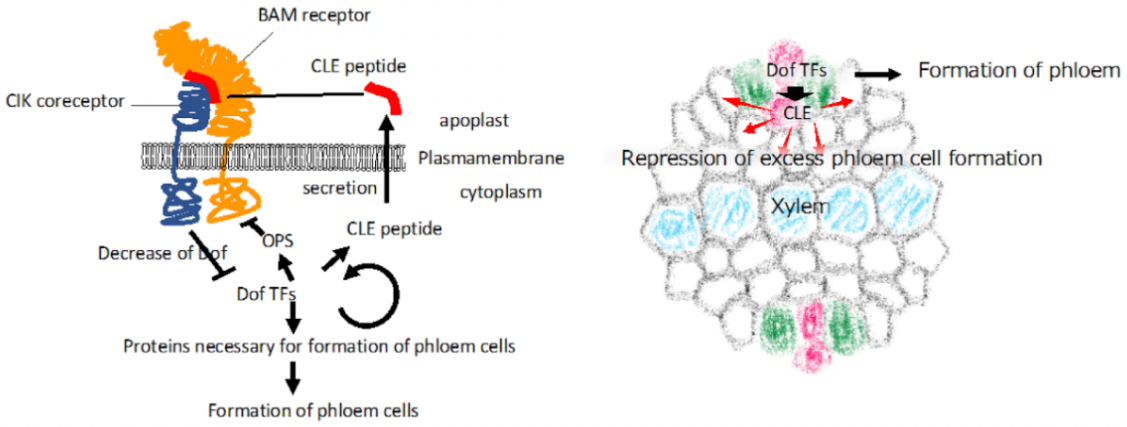

Figure 1: Phloem formation depends on interconnected positive and negative regulation.

Poster 20

PENGBO LIANG

China Agricultural University, Beijing

Symbiotic intracellular infections in legume

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To achieve successful intracellular infection, legumes gain specific invention to recognise rhizobia via Nod Factor receptors and root hair curling to entrap the symbiont. The entrapment of the symbiont completes with its full enclosure in a structure called the ‘‘infection chamber’’ (IC), from which the intracellular infection initiates, to form a tube-like membrane channel ‘‘infection thread’’ (IT). Previously, we uncovered receptor LYK3 stabilization is mediated by remorin protein SYMREM1 in the nanodomain, which is required for its function. In addition, a formin protein, SYFO1, mediated cell wall-plasma membrane-cytoskeleton continuum is required for root hair deformation and curling.

Here, we identified a new formin protein SYMBIOTIC FORMIN 3 (SYFO3) in *Medicago truncatula*, which is essential for symbiotic interactions. SYFO3 was transcriptionally upregulated upon rhizobia inoculation and directly targeted by transcription factor NIN. Homogeneous plasma membrane localized SYFO3 displayed two distinguished localisation fashions upon inoculation of rhizobia overtime, which were punctate and mixed punctate-filament. The triggered punctate localized SYFO3 resembled a nanodomain distribution pattern and co-localised with SYMREM1, and later form network has a spoke-and-hub organization, where the hub maintained the colocalisation with SYMREM1. Furthermore, FLIM-FRET data indicated SYFO3 interacted with SYMREM1 *in vivo*.

Interestingly, phylogenetical analysis supported that SYFO3 exclusively belongs to Arbuscular Mycorrhiza Fungi (AMF) infection clade. Its promoter activity was specifically triggered by AMF infected or adjacent cells, and SYFO3 localized around the periphery of freshly branched arbuscules. The number of AMF infection compromised in *syfo3* mutants. The above two impaired infection phenotype suggests a global role of SYFO3 in microbe intracellular infection.

Poster 21

BRIDGET HUA BAI

University of California, San Diego, USA

Unbiased RNA and protein co-expression networks highlight important regulatory role of organelle protein homeostasis in maize heterosis

Bridget Hua Bai, Devon Birdseye, Zhouxin Shen, Steve Briggs

University of California, San Diego

Heterosis, or hybrid vigor, is the difference in vigor between a hybrid and the average of its parents. Crops and livestock have been bred as hybrids for decades for increased vigor from hybridity, yet its underlying molecular mechanism remains elusive, and the ability to leverage the driving force of heterosis will have great impact in agriculture. Our recent published work showed that the abundance of photosynthesis related protein complexes is elevated in the hybrids relative to mid-parent levels, suggesting greater photosynthetic capacity. Yet no connections were made with other pathways that could explain this observation. To identify key drivers of heterosis, we constructed RNA and protein co-expression networks by Weighted Gene Co-expression Network Analysis (WGCNA) using transcriptomics and proteomics data collected from maize inbred and hybrid leaves. Both networks revealed a set of organelle protein homeostasis (proteostasis) candidates with high positive correlations to a key heterotic trait, plant height heterosis (hybrid/mid-parent plant height). Proteostasis candidates included proteases, chaperones, chloroplast translocons (TOC/TIC) components, proteasome subunits and more, many of which are upstream and indispensable for photosynthesis. Increased abundance of photosynthesis protein complexes could be explained by higher protein folding and degradation efficiency from elevated expression of chloroplast proteostasis machinery subunits in the hybrids. We also found that bzip60, an unfolded protein response (UPR) hallmark in plants, was a key negative regulator of heterosis. Comparison of transcript levels showed that most hybrids had below mid-parent expression of bzip60, and highly heterotic hybrids expressed bzip60 below low-parent levels. This suggested that inbreds induced more UPR, possibly due to lower ER stress tolerance and more protein aggregation compared to the hybrids. Elevated expression of UPR transcripts in the inbreds was also observed in non-green tissues, indicating that greater proteostasis stress tolerance in the hybrids may contribute to heterosis in both green and non-green tissues. Our results provided strong explanations for observed molecular phenotypes and established the important regulatory role of organelle proteostasis in causing hybrid vigor.

Poster 22

CORAL MARTÍNEZ

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Tissular and Subcellular Localization of a Protein Induced by Water Deficit in Arabidopsis Thaliana: The case of AtLEA4-5 Protein

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Late Embryogenesis Abundant (LEA) proteins accumulate during the last stages of seed development and in vegetative tissues in plants under water deficit. Most of the LEA proteins are considered intrinsically disordered proteins (IDPs). Previous work uncovered the relevance of group 4 LEA proteins in plant tolerance to water deficit. In *Arabidopsis thaliana* this group is formed by AtLEA4-1, AtLEA4-2 and AtLEA4-5, and mutant plants in any of these members are more susceptible to water deficit; also, *in vitro* assays demonstrated that AtLEA4-2 y AtLEA4-5 proteins could protect the activity of reporter enzymes when they were subjected to partial dehydration or freezing/defrosting cycles. This evidence indicate that these proteins could be exerting a protective function in the plant against to water deficit; nevertheless, little is known about if their function in the plant is limited to certain tissues and cellular compartments. In this project, we address the localization of AtLEA4-5 protein, since is one of the most studied members of group 4. Using fusions to GFP, so far, we know that AtLEA4-5 protein is localized in all the tissues of imbibed seeds and apparently this localization is maintained during the first days after germination. In seedlings expose to salt, this protein localizes in vascular tissues from roots. At subcellular level this protein localizes in nucleus and cytoplasm, but it remains to be determined if localization in cytoplasm corresponds to a certain(s) organelle(s). The information obtained from this analysis could indicate to us where this protein carries out its function(s) in the plant and suggest to us which biological/molecular processes are involved.

Poster 23

KAREN SERRANO

Joint BioEnergy Institute, USA

Mapping multi-kingdom symbiotic interactions with spatial transcriptomics and single-nucleus sequencing

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The interaction of plants with arbuscular mycorrhizal fungi (AMF) is both ancient and widespread. In this symbiotic relationship, plants (including the legume model, *Medicago truncatula*) provide AMF with carbon in exchange for phosphorus, nitrogen and water, making this interaction a prime target for crop improvement. Despite decades of research, we still lack a comprehensive understanding of the molecular features involved in establishing and maintaining AMF symbiosis. This is in part due to the difficulty of studying interactions that occur only in a limited set of cells within plant roots, precluding many powerful functional genomics strategies. To overcome this hurdle, we have piloted the use of two recently developed and highly complementary transcriptomics methods: single-nucleus sequencing and spatial transcriptomics. Integration of these data types enables the creation of spatially-registered single-cell maps of the transcriptomes of both plant and fungal cells during colonization. We have identified most major cell types in *M. truncatula* root, including subclusters of cortical cells at different stages of colonization by AMF. Transcriptome analysis of colonized cortical cells revealed multiple genes were not previously known to be involved in this interaction. We are now applying this technique to *M. truncatula* mutants to determine critical genetic pathways that can be exploited to engineer this interaction.

Poster 24

MUSKAN KAPOOR

Iowa State University, USA

Creating a FAIR data ecosystem for incorporating single cell genomics data into agricultural G2P research

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The agriculture genomics community has numerous data submission standards available, but little experience in describing and storing single cell (e.g., scRNAseq) data. Other single cell genomics infrastructure efforts, such as the Human Cell Atlas Data Coordination Platform (HCA DCP), have resources that could benefit our community. For example, the HCA DCP is integrated with Terra, a cloud-native workbench for computational biology developed by Broad, Verily and Microsoft that houses tools for scGenomics analysis. We will describe a pilot-scale project to determine if our current metadata standards for livestock and crops can be used to ingest scRNAseq datasets in a manner consistent with HCA DCP standards and if established resources (e.g., Terra) can be used to analyze the ingested data. Currently, the most comprehensive data ingestion portal for high throughput sequencing datasets from plants, fungi, protists, and animals (including human) at the EMBL-European Bioinformatics Institute, Annotare, ensures that sufficient metadata are collected to enable re-analysis and dissemination via the Single Cell Expression Atlas (SCEA). Annotare supports an auto-complete function that allows the users to search for and use the appropriate terms from many ontologies and can readily be used to process and search single cell plant data via the SCEA, as well as transferred to the Galaxy analysis space. All experiments submitted to ArrayExpress via Annotare are manually curated by bioinformaticians. Another EMBL-EBI portal that is limited to animal datasets, the FAANG portal, provides access to bulk and scRNAseq data. scRNAseq data/metadata can be submitted to the FAANG portal using a semi-automated process where files can be validated using the HCA DCP metadata and data validation service. Once incorporated, datasets are used to augment the DCP resource for the scientific community. These files are also incorporated using EMBL-EBI's HCA DCP ingestion service, and then transferred to Terra for further analysis.We intend to build upon these existing tools to construct a scientist-friendly data resource and analytical ecosystem to facilitate single cell-level genomic analysis through data ingestion, storage, retrieval, re-use, visualization, and comparative annotation across agricultural species.

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Poster 25

DAE KWAN KO

Michigan State University, USA

Network-enabled regulatory dissection of the mixed-linkage glucan synthase genes in grasses

Mixed-linkage glucan (MLG) is a highly abundant polysaccharide in the cell walls of grasses with potential for biomass improvement. Since MLG is easily fermentable, it is ideal for the production of biofuels and glucose-derived bioproducts. Therefore, bioenergy crops with a high amount of MLG are likely valuable as industrial feedstocks. However, how CSLF6 genes are regulated in grasses is poorly understood. Such knowledge could allow for engineering bioenergy crops with high levels of MLG. To address this gap, we performed coexpression-based gene regulatory network analyses using time-series transcriptome datasets spanning the entire life cycle of sorghum and Brachypodium to identify coexpression modules and transcriptional regulators of CSLF6 in each species. The network analyses predicted candidate transcription factor genes for CSLF6 regulation based on the expression timing and strength within the coexpression module in each species. Through promoter analyses, we discovered conserved and diverged regions of CSLF6 promoters across grasses, pinpointing cis-regulatory elements for regulating CSLF6 genes. Together, our approach not only revealed the regulatory dynamics of CSLF6 genes during the development but also provided cross-species predictions for trans- and cis-acting factors in regulating CSLF6 genes.

Poster 26

MARTA PEIRATS-LLOBET

La Trobe University, Australia

Mapping Cell-Specific Activity During Germination Of Barley Grain Using Spatial Transcriptomics

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Seeds provide 70% of global food resources, being the most valuable output from plant production. They also play a critical role in agriculture because the lifecycle of most crops begins from seed germination. Uniform germination enables growers to achieve optimal plant-spacing and harvesting time. Despite this importance, we do not have a complete understanding of how seed germination is regulated, which limits our ability to improve its properties. We have made much progress identifying regulators by traditional bulk-tissue 'omics approaches. Through these we have discovered transcription factors that control both gene expression and the progress of germination. However, the seed is a complex structure comprised of many tissues and cell-types, each of which have distinct properties. Changes in gene expression occur in these cell-types throughout germination and are expected to be context-dependent, to enable spatiotemporal control of cellular processes. To better understand how gene expression is controlled within individual seed cell-types and tissues we have carried out spatial transcriptomics in germinating barley grains. This approach allows us to assay gene expression on a high-resolution spatial map of the grain. Using time-series analyses we are investigating spatiotemporal regulation of gene expression as germination progresses. This will help us to develop practical solutions to promote the seed-to-seedling transition and to ensure germination happens uniformly at the right time.

Poster 27

VIMAL KUMAR BALASUBRAMANIAN

Pacific Northwest National Laboratory, USA

Cell-type Specific Responses of Poplar to Stress Combination: An Integrated Network Assisted Proteomics and Spatial Metabolomics Approach

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Combination of multiple abiotic stressors significantly decrease the plant productivity and overall health. Many studies have shown that the response of plants to stress combination is unique and notably different compared to individual stresses. Moreover, plant responses to stress involve complex crosstalk between different regulatory pathways, including metabolic adjustments and gene/protein expression at cellular levels. However, current knowledge in plant molecular responses to stress combination mainly relies on omics approaches conducted at the tissue levels, primarily due to the lack of reliable and accurate spatially-resolved and cell type-specific multi omics platforms for plant systems. In this work, using laser capture microdissection (LCM) based proteome analysis coupled with a spatial metabolomics approach, we aimed to discover the underlying molecular mechanisms of distinct cell-types of leaf tissue regulating plant developmental processes and plasticity when grown under combined abiotic stress conditions. For that, poplar trees exposed to salinity, heat, water deficit, and combination of three stresses were used. Our cell-type proteomics analysis was enabled by a novel microfluidic nanodroplet-based sample preparation technology (nanoPOTS) coupled with a highly sensitive mass spectrometry. Using this approach, we reliably detected a total of 6,834 proteins from leaf palisade and vascular cell types isolated from controls, single stress, and triple stress combinations. Among the identified proteins, 10.9% (palisade) and 45.2% (vascular) of proteins were unique to leaf cell types, while 43.7% of proteins were commonly expressed in both cell types. To identify proteins with possible key roles in plant stress response in each cell types, we used a multi-faceted approach that detected proteins with 1) significant relative abundance levels during stress, and 2) high network centrality in a protein co-abundance network. Therefore, we identified several unique combined stress responsive proteins (compared to individual stresses) in palisade and vascular cell-types. To link the protein abundance profiles with the spatial distribution of associated metabolites, we used matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) and mapped the relative abundance of metabolites in different cell-types during stress combination. Interestingly, we found positive correlations between the accumulation of specific metabolites involved in the glycolysis/gluconeogenesis pathway and their associated protein abundance levels exclusively in palisade cells, highlighting the cell-autonomous regulation of central carbon metabolism in palisade cells under the simultaneous occurrence of salinity, heat and water deficit stresses. Overall, our findings highlight the spatial distinction in stress-responsive mechanisms and allow future attempts in mapping molecular machineries to the cellular domain and contribute to the design of plants with enhanced tolerance to abiotic stress combination.

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