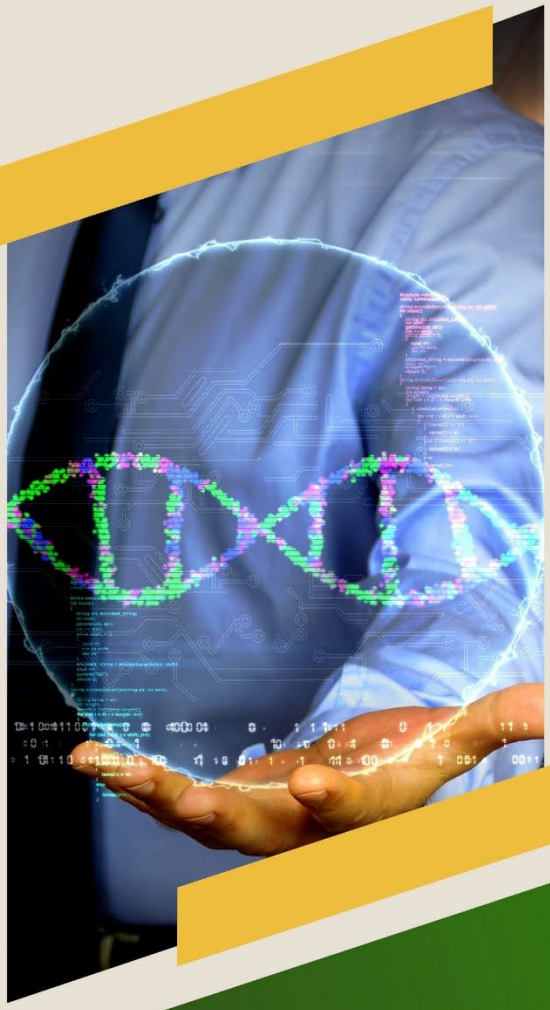
SKLA/IPMBAB Seminar Series 2024



Date: 9 November 2024 (Sat)
Venue: LT5, Yasumoto International Academic Park, CUHK

Abstract Booklet

DNA hypomethylation is one of the epigenetic mechanisms involved in salt-stress priming in soybean seedlings

Long Yiu CHAN and Hon-Ming LAM

State Key Laboratory of Agrobiotechnology (CUHK) and

School of Life Sciences, The Chinses University of Hong Kong

Speaker

1

Environmental stresses are severely threatening global food security. Soybean, as one of the major crops, is sensitive to salt stress. Through salt stress priming, primed soybean that is pre-treated with mild salt gain enhanced tolerance to subsequent high salt stress. Multiple epigenetic factors, which mediate the transcriptomic reprogramming for more effective stress responses, are previously reported to be involved in stress priming in plants.

In this study, it is found that salt stress priming in soybean was featured by an overall DNA hypomethylation in leaves, as indicated by the whole-genome bisulfite sequencing data. Genes associated with DNA hypomethylation regions in primed soybean (hypo-DMGs) also showed a higher mean level of the active histone mark H3K4me3 and a lower mean level of the repressive histone mark H3K4me2. Further analysis of the transcriptomic profile supported that DNA hypomethylation played a role in finetuning the chromatin statuses to potentiate gene expression in primed soybean. Motif analysis found that an ABA-responsive element (ABRE)-like motif was significantly enriched in the promoter regions of the triggering stress-induced hypo-DMGs. Transcriptional network analysis also revealed that DNA hypomethylation may facilitate the transcriptional responses mediated by key transcription factors in the ABA-related pathway. The pre-treatment using a DNA methyltransferase inhibitor, 5-Azacytidine, could enhance the tolerance of soybean seedlings to subsequent high salinity, which further supported the importance of DNA hypomethylation in salt stress priming in soybean.

Comparative GC-MS and LC-MS/MS Analysis of Terpenoids, Fatty Acids, and Flavonoids in Diverse Soybean Germplasms

Runmeng HU, Xia ZHANG, and Pan LIAO

State Key Laboratory of Agrobiotechnology (CUHK) and

Department of Biology, Hong Kong Baptist University

Speaker

2

Soybeans are known for their rich bioactive compounds, which contribute significantly to human health. Soybean seeds from different genetic backgrounds and geographical regions exhibit distinct profiles of secondary metabolites influenced by various selection pressures. We hypothesize that specific soybean germplasm resources contain higher levels of beneficial metabolites, such as flavonoids and terpenoids, which may confer additional health benefits and potential insect resistance. To test this, we employed GC-MS to analyze terpenoids and fatty acids, while utilizing LC-MS/MS for flavonoid quantification. The results revealed significant variations in metabolite levels across the different soybean varieties. Notably, certain germplasms demonstrated elevated abundance of secondary metabolites linked to anti-cancer and anti-inflammatory properties. The identification of these high-quality soybean germplasm resources could pave the way for breeding programs focused on enhancing nutritional quality and pest resistance. In conclusion, this research underscores the importance of metabolite profiling in soybean germplasms, providing a foundation for future studies aimed at improving soybean quality and nutrition through targeted breeding strategies.

Sources of cytosolic NADPH in *Arabidopsis thaliana*

Danying LU and Boon Leong LIM
School of Biological Sciences, the University of Hong Kong

Speaker
3

NADPH is a ubiquitous energy carrier in all living organisms and is essential for maintaining redox homeostasis and various metabolic pathways. In plants, NADPH is produced by various NADPH-producing enzymes in different subcellular compartments.

Cytosol is a crucial compartment of plant cell that contributes to NADPH regulated redox homeostasis and lipogenesis. There are multiple NADPH-generating enzymes in the cytosol of Arabidopsis, including glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the oxidative pentose phosphate pathway, NADP-dependent isocitrate dehydrogenase, NADP-dependent malic enzymes and non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase. Among these, which enzyme(s) is/are the main source(s) of cytosolic NADPH in various cell types remains unknown.

Previous methods for measuring metabolite levels in plant cells were mostly indirect and required the extraction of these metabolite before measurements, making it inaccurate and impossible to observe dynamic changes in the subcellular levels of these molecules. In this study, by introducing a novel fluorescent energy sensor mcherry-iNAP1 into several Arabidopsis mutants, we will be able to monitor the dynamic changes of cytosol NAPDH levels in different tissues of these mutants. In addition, the promoter of each enzyme will be fused with a GUS vector and the vectors will be transformed into wild type Arabidopsis. Then, the expression pattern of each gene in different tissues of Arabidopsis will be visualized by GUS histochemical staining. Through the combination analysis of the GUS data of each gene and the NADPH levels in different tissues of their corresponding mutants, this study aims to clarify the main sources of NADPH in different tissues in Arabidopsis.

Future CO_2, Ozone, and Climate Change Impacts on Terrestrial Productivity

Hongru WANG and Amos Pui Kuen TAI

Department of Earth and Environmental Sciences

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Speaker

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Terrestrial gross primary productivity (GPP), a key component of ecosystem functioning, is influenced by environmental factors like atmospheric CO_2, tropospheric ozone (O_3), and climate change. While individual impacts of these factors have been widely studied, their interactive effects remain less understood, varying by region and season due to environmental conditions and plant traits**.** In this study, we use factorial model experiments to quantify the individual and synergistic effects of future atmospheric CO_2, O_3, and climate change on GPP during 2055-2061 under the Shared Socioeconomic Pathway SSP3-RCP7.0 scenario, using the lightweight land ecosystem model, Terrestrial Ecosystem Model in R (TEMIR). By evaluating the impacts of these factors on GPP with both present-day Leaf Area Index (LAI) and projected future LAI, we distinguish between direct environmental effects and the indirect influences mediated by changes in vegetation structure on future GPP dynamics.

Our results demonstrate that CO_2 fertilization significantly enhances global GPP by 21 Pg C yr^{-1} (+14%), whereas future O_3 damage reduces GPP by 5.4 Pg C yr^{-1} (–3.6%), exceeding climate change impacts (–2.6 Pg C yr^{-1}, –1.8%). The anticipated future increase in O_3 concentration lead to 1.5 times present-day O_3 damage. Climate change mainly reduces GPP in the Southern Hemisphere due to changes in temperature and moisture. Notably, while global LAI is projected to increase, this may lead to a counterintuitive reduction in GPP due to disproportional regional GPP responses to LAI changes, influenced by regional LAI-light saturation variations that limit light-use efficiency through self-shading.

Regarding factor interactions, CO_2 fertilization effectively reduces stomatal conductance more than O_3 damage, mitigating future O_3 damage by ~20%. Interactions among future CO_2, O_3, and climate change enhance GPP in most regions. Under future LAI, these interactions also generally increase GPP, while future LAI and climate-induced reductions may outweigh the CO_2 fertilization effect, leading to a net GPP reduction in temperate Africa, arid Africa, and arid Oceania, necessitating further attention. Elucidating these impacts enhances our knowledge of ecosystem responses and helps develop effective strategies to address climate change, O_3 pollution, and other environmental stressors impacting global terrestrial productivity.

A phase-separating mRNA-binding protein enhances drought stress tolerance by stabilizing *GAD2* transcripts in *Arabidopsis*

Chuangfeng LIU and Liming XIONG

Laboratory of Plant Stress Biology

Department of Biology, Hong Kong Baptist University

Speaker

5

mRNA-binding proteins (mRBPs) play essential roles in every step of mRNA metabolism. In the *Arabidopsis* genome, hundreds of mRBPs are identified yet the functions of most of these proteins are largely unknown. In this study, we characterized a HABP4 type mRNA-binding protein HLN1 and found that HLN1 forms mRNA-ribonucleoprotein (mRNP) condensates in the cytoplasm under osmotic stress conditions. Transcriptomic analysis revealed that the knockout of *HLN1* leads to altered the expression of genes in the pathways of carbohydrate metabolism and biosynthesis of secondary metabolites. Among these genes is the glutamate decarboxylase 2 (*GAD2*) that encodes a key enzyme in the synthesis of gamma-aminobutyric acid (GABA), a novel signaling molecule that regulates stomata movement. Using RIP-qPCR and EMSA analysis, we verified the interaction between HLN1 and *GAD2* mRNA *in vitro* and *in vivo*. In *hln1* mutants, *GAD2* transcripts are less stable, resulting in reduced steady-state mRNA levels than in the wild type plants. Compared with the wild type, *hln1* mutants also accumulate lower levels of GABA and their stomata closure was impaired in response to drought stress. Overexpression of *HLN1* stabilizes *GAD2* mRNA, increases GABA levels, and improves drought tolerance of the transgenic plants. Taken together, our study uncovers a mechanism whereby HLN1 stabilizes *GAD2* mRNA and increases the production of GABA under drought stress and thus enhances plant tolerance to drought stress. Supported by UGC/RGC GRF #12103020 and NSFC/RGC/CRS CRS_HKBU201/22.

A gene regulatory network for Casparian strip formation in maize root exodermis

Weilun LIU and Silin ZHONG

State Key Laboratory of Agrobiotechnology (CUHK) and

School of Life Sciences, The Chinese University of Hong Kong

Speaker

6

Transcription Root architecture is optimized to selectively absorb nutrients and water, while protecting against toxic compounds and pathogens. This selectivity t is largely depend on the formation of an apoplastic diffusion barrier, called 'Casparian strips' (CSs). For most plants, the CSs are found in the root endodermis, an inner layer of cells that surrounds the vascular bundles. Past studies in the model plant Arabidopsis have identified several important genes of CSs formation in endodermis. The CASPARIAN STRIP MEMBRANE DOMAIN PROTEINS (CASPs) are acting to bring together NADPH oxidase and peroxidase, allowing localized ROS production toward peroxidases, thus ensuring localized and efficient lignification. These genes are under transcriptional controls of a network of TFs including SHR, SCR and MYBs in accordance with endodermis development and in response to environmental stresses.

Unlike Arabidopsis, many plant species such as rice, tomato and maize have a more complex root architecture with an extra specialized cell layer, called exodermis. Exodermis is a cell layer outside the cortex and underneath the epidermis, and it can also form CSs. In maize, the exodermis demonstrated great development plasticity and has been shown to be related with stress tolerance. However, the development pathways for exodermis and its CSs formation regulation remain uncharacterized. Our analysis of previously published scRNA-seq of maize root identified endodermis and exodermis cell clusters and the genes specifically expressed in them. It showed that different sets of CASPs were expressed in endodermis and exodermis suggesting that these two types of cells utilize different sets of genes to construct CSs. Using protoplsat transformation, a scalable and fast ChIP-seq method for mapping genome-wide binding sites was developed and applied to 14 exodermis-TFs. By inferring their target genes, these TFs are shown to play important and diverse roles in the lignin biosynthesis and Casparian strip formation. Next, ChIP-seq will be applied to more exodermis-TFs to identify more TFs regulating Casparian strip formation and construct a transcriptional network for it.

Identification and characterization of new regulators in regulating vacuole dynamics and vacuolar transports via the brassinosteriod signaling pathway

Chudi Fan and Liwen JIANG

Centre for Cell & Developmental Biology and State Key Laboratory of Agrobiotechnology, School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, China

Speaker

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Vacuole is one of the important membrane-bound organelles in the plant endomembrane system, including the endoplasmic reticulum (ER), Golgi apparatus, trans-Golgi network (TGN), prevacuolar compartment (PVC) or multivesicular body (MVB) and vacuoles. Plants maintain the physiological homeostasis through vacuole dynamics to control the cell size in response to environmental stimuli or hormone. Multiple regulators have been suggested to play roles in regulating vacuole dynamics during plant growth and development.

Brassinosteriods (BRs) are a class of steroid phytohormone involved in regulating the plant cell division, elongation, and differentiation in different tissues and cell types through the BR signaling pathway. Our preliminary data showed that vacuole dynamics respond to BR signaling in *Arabidopsis* seedlings, and we have thus speculated that vacuole morphological changes affecting root cell development are regulated by the BR signaling. Here we aim to further identify novel regulators in the BR signaling pathway for vacuole dynamics and plant growth. Our preliminary results uncovered the impact of BRs on the shapes of vacuoles. Confocal imaging analysis revealed that vacuoles in root cells of *Arabidopsis* seedlings became rounded or fragmented with exogenous 24-Epibrassinolide (eBL) and brassinazole (BRZ) treatments. These results were further confirmed by phenotypic analysis of various *Arabidopsis* mutants of BRASSINOSTEROID INSENSITIVE1 (BRI1), BRASSINOSTEROID-INSENSITIVE 2 (BIN2), and BRI1-EMS-SUPPRESSOR 1(BES1), which are key regulators of BR signaling, suggesting that vacuole dynamics are related to BR signaling. We speculated that BR post-translationally regulates the MVB-vacuole fusion that affects the vacuole dynamics. To further investigate the underlying mechanisms of vacuole morphological changes, we have identified FREE1 (FYVE domain protein required for endosomal sorting 1) as key regulator that may regulate the MVB-vacuole fusion through BR signaling pathway. We performed yeast-two hybrid (Y2H) and co-immunoprecipitation (Co-IP) to test that FREE1 interacts with BIN2 both in vitro and in vivo. The interaction between BIN2 and FREE1 prompted us to hypothesize that BIN2 might phosphorylate FREE1 to regulate MVB-vacuole fusion during eBL treatments that would be further confirmed by experiments.

The roles of protochlorophyllide oxidoreductases (POR) and FZO-like protein (FZL) in *Arabidopsis* prolamellar bodies (PLB) biogenesis

Man Ip HO and Byung Ho KANG

School of Life Sciences, The Chinese University of Hong Kong

Speaker

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The chloroplast is a type of plastid that contains thylakoid membranes where the photosynthesis light reaction occurs. When proplastids in seeds are exposed to light, they develop into chloroplasts and acquire thylakoids. In the absence of light, the proplastid-to-chloroplast development is arrested to form etioplasts that quickly transform into chloroplasts once light becomes available. They contain semi-crystalline membrane tubules termed the prolamellar body (PLB) that stores proteins, lipids, and chlorophyll precursors to construct thylakoids.

Extensive research has been carried out to understand the molecular mechanisms that underlie photomorphogenesis and the etioplast-to-chloroplast transition. During photomorphogenesis, the tubulovesicular membranes in proplastids increase in size and merge into a complex membrane network consisting of stroma thylakoids and grana stacks. CURT1 family proteins along with a dynamin-related protein called FZL play essential roles in the membrane dynamics that are involved in chloroplast biogenesis. CURT1 proteins oligomerize and bind to the highly bent thylakoid membranes at the grana margins to stabilize them. FZL mediates the fusion of thylakoid membranes to construct a continuous thylakoid network. POR proteins accumulate in the PLB during angiosperm skotomorphogenesis.

Currently, our knowledge regarding the molecular factors and regulatory processes involved in the development of etioplasts during skotomorphogenesis is limited. We investigated the assembly process of PLBs using three-dimensional electron microscopy (3D EM) and examine the functions of proteins associated with PLBs. Our initial findings indicate that bubbles are trapped in assembly intermediates of PLBs in *pora* mutant etioplasts. Smaller PLB and bubbles between tubular structures outside the PLB are observed in *porb* mutant etioplasts. In *porab* mutant etioplasts, more tubular structures are observed and it has a low chance to observe a PLB. PLB tubules form bundles instead of the regular hexagonal arrangement in *fzl* etioplasts indicating that FZL-mediated membrane fusion is requied for the PLB assembly.

Impact of *Lhx1/5* Inactivation on Purkinje Cell Function: Ataxia and Disruption of Neurotransmitter Homeostasis

Le SHEN and Kin Ming KWAN

State Key Laboratory of Agrobiotechnology (CUHK) and

School of Life Sciences, The Chinese University of Hong Kong

Speaker

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Purkinje cells (PCs) play a crucial role in cerebellar function, contributing significantly to motor coordination and the precise tuning of movements. Our previous work involved characterizing an ataxia mouse model, the *Pcp2-cre Lhx1/5* double conditional knockout (DKO) mutant mouse, wherein we identified morphological and electrophysiological defects in PCs.

In our current study, we have discovered that the expression level of the PC-specific excitatory amino acid transporter EAAT4, which is essential for clearing excess glutamate from the synaptic cleft, is notably reduced in these mutant mice. This dysregulation potentially caused excitotoxicity and altered synaptic signaling. Besides, EAAT4 exhibits a distinct expression pattern in the *Lhx1/5* DKO mice cerebellum, with a more severe loss of EAAT4 expression in lobules I-VI. These lobules are primarily involved in fine motor control, sensory integration, and cognitive functions, whereas lobules VII-X specialize in balance and vestibular processing.

Furthermore, our study revealed significant changes in the expression of various neurotransmitter receptors in the cerebellum of *Lhx1/5* DKO mice, suggesting a broader disruption of neurotransmitter homeostasis. This imbalance contributed to altered synaptic signaling and impaired electrophysiological properties of Purkinje cells.

These molecular changes were linked to compromised Purkinje cell function, as evidenced by impaired electrophysiological properties and reduced motor coordination. Overall, this study enhances our understanding of the molecular basis of cerebellar dysfunction and suggests potential therapeutic strategies for conditions such as ataxia.

Investigation on the Role of Macrophage and Mitochondria in Mediating Breast Cancer Progression

Yan Wah CHONG and Suk Ying Faye TSANG

State Key Laboratory of Agrobiotechnology (CUHK) and
School of Life Sciences, The Chinese University of Hong Kong

Speaker

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Cell fusion is a process in which two or more cells merge their plasma membrane and nucleus into one. It has been considered a driving force of tumor progression contributing to aneuploidy, cancer stem cell development and metastasis. Being a solid tumour, the interplay among tumor cells, stroma, and immune cells in the complex tumor microenvironment of breast cancer is particularly important, which could impact cancer progression and treatment outcomes. Among the extensively researched tumor-infiltrating immune cell groups are tumor-associated macrophages, which are associated with poor prognosis in breast cancer patients. More studies have now highlighted the role of cell fusion between breast cancer cells (BCCs) and macrophages in cancer progression, which enhanced aggressiveness was observed in hybrids. Previous study by our group has unveiled the ability of cancer cells to reverse the programmed cell death apoptosis and become more aggressive. The role of mitochondrial processes in cancer progression has been controversial, with evidence that they can both suppress and promote tumour growth depending on the stage of the cancer.

We hypothesize that fusion between macrophage and BCCs could be affected by apoptosis reversal, and the fusion process promotes cancer progression through reshaping the mitochondrial physiology of the hybrids. In this proposed study, we aim to investigate the fusion between macrophages and breast cancer cells, particularly whether macrophages have a higher propensity to fuse with apoptosis-reversed breast cancer cells. We also aim to determine if the resulting fusion hybrids exhibit enhanced aggressiveness, and if so, whether changes in mitochondrial processes contribute to this increase.

Our result showed successful differentiation of monocytic cell line into macrophages by phorbol 12-myristate 13-acetate (PMA) treatment. Primary macrophages were also derived successfully from mouse bone marrow. Preliminary imaging data demonstrated cell fusion between BCCs and macrophages. Flow cytometry analysis revealed that the fusion efficiency between reversed BCCs and macrophages was significantly higher than that between control BCCs and macrophages. The project will proceed to investigate and compare the aggressiveness of resultant hybrid between the groups and the role played by mitochondrial processes.

Characterization of autophagy-mediated organelle homeostasis in green alga *Chlamydomonas reinhardtii*

Yixin Wu and Xiaohong ZHUANG

School of Life Sciences, Centre for Cell and Developmental Biology, State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Hong Kong, China.

Speaker

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Investigating how autophagosome recognizes and contacts with the targeting organelles is crucial for understanding the impacts of autophagy-dependent metabolism on organelle homeostasis, cell physiology and fitness. However, the cellular events and the molecular network that governing the selective degradation of specific organelle are still poorly characterized in green organisms, especially in regarding to the molecular regulatory hierarchy. During autophagy, autophagosome formation is the hallmark of autophagy in most eukaryotic cells, which involves a key regulator named autophagy-related (ATG) 8. In yeast and mammalian cells, ATG8 protein family has been reported to exert multifunctional roles during autophagy. By far, the impact on autophagic activity and autophagosome biogenesis when loss of ATG8 proteins has not been evaluated in detail in plant systems, leading to the physiological function of plant ATG8 proteins remains elusive. Unlike the higher plants that contain multiple copies of ATG8, there is only a single copy of ATG8 in the unicellular green alga *Chlamydomonas reinhardtii*. The biochemical property of ATG8 proteins and ATG8 conjugation system in Chlamydomonas has been well characterized. However, the interplay between autophagosome and other organelles remains largely unexplored. Here we have employed the CRISPR-Cas9 system to generate specific Chlamydomonas mutants, and have characterized the interplay between autophagosome and cellular compartments under different stress conditions in Chlamydomonas cells.

Genomics study of underutilized legumes to accelerate orphan crop improvement

Tsz Yan CHEUNG and Ting Fung CHAN

State Key Laboratory of Agrobiotechnology (CUHK) and

School of Life Sciences, The Chinese University of Hong Kong

Speaker

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The escalating global population, combined with increasing food demand under a changing climate condition, poses a significant threat to global food security, especially in underdeveloped countries. Staple crops currently contribute to the major part of food security, however, their yield are largely affected by climate change. In contrast, underutilized crops are neglected crops growing in localized areas with corresponding stress tolerance, holding potential for sustaining food production against climate change. Nonetheless, the lack of genomic resource and biological information has restricted the crop improvement of these underutilized crops, limiting the efforts to develop varieties with high yield, nutritious value and stress resilience.

In this study, three underutilized legumes, namely *Lablab purpureus*, *Macrotyloma geocarpum* and *Vigna vexillata*, have been selected as our crops of interest. To explore their untapped potential, we have constructed the first high-quality chromosome-scale genome assemblies for the wild accession of the three selected legumes. Comparative genomic analyses are performed to examine the genomic differences underlying wild and domestication. With high-quality genome assemblies as references, multiple wild and domesticated accessions have been re-sequenced for population analysis, aiming to identify genes associated with agronomic traits and possible biological pathways involved in domestication. To capture the full spectrum of genomic and genetic diversity within the species, we have particularly selected several representative accessions from *L. purpureus* for whole-genome long-read sequencing and constructed a pan-genome. One of our results demonstrates the exceptionally low genomic diversity in *M. geocarpum*, approximately 95% less than that observed in *L. purpureus*, suggesting an urging need to conserve the existing diversity and expedite efforts on other underutilized legumes.

Altogether, our study could provide genomic basis for comprehensive investigations into the diversity between gene pools and generate valuable genetic resources which could be used in future breeding programmes, ultimately accelerating the crop improvement of these neglected crops and thereby increasing utilization of crops to achieve food security under climate change.

Dynamic DNA methylation during early embryogenesis in *Arabidopsis*

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Speaker

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DNA methylation is one of the well-studied epigenetic modifications that has impacts on the expression of genes and transposable elements (TEs). In plants, DNA methylation on cytosines can be detected in the contexts of CG, CHG and CHH, where H represents A, T, or C. Embryogenesis is a crucial developmental process in the life cycle, accompanied by the complex pattern formation and morphogenesis. During early embryogenesis in *Arabidopsis*, maternal and paternal genomes contribute equally to the embryonic transcriptome initiating from 1/2-cell stage. Recently, research efforts have been dedicated to investigating the remodeling of DNA methylation after fertilization. In *Arabidopsis*, gain of CHH methylation is found since globular embryo during seed development. In rice zygote, DNA methylation of paternal allele undergoes remodeling to match that of maternal allele. The essential roles of DNA methylation have been illustrated by the extreme developmental defects in DNA methylation-free *Arabidopsis*. However, limited by the challenges of isolating embryos without contamination, knowledge about epigenetic regulation during early embryogenesis remains limited in *Arabidopsis*.

In this study, we manually isolated *Arabidopsis* early hybrid embryos to perform bisulfite sequencing and RNA-seq. Our results uncovered the dynamic changes of DNA methylation during early embryogenesis, represented by the remarkable gain of CHH methylation and the progressive loss of CHG methylation. Genes with differential methylation were found to be associated with morphogenesis, pattern specification and auxin transport. The integrated analysis with embryonic transcriptome data suggested the relevance between CHH hyper-methylation and TE repression in globular embryo. Moreover, after assigning the parental origins of reads, variations between allelic methylation were explored. A negative relevance between allele-specific expression and methylation at CHG sites was found in early developing embryos. Together, this work provided new insights into the remodeling of DNA methylation and its regulatory role during early embryogenesis in plants.

Developing Methods for Spatial Omics Analysis in Plants

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Speaker

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High-resolution spatial omics, encompassing in-situ-capture transcriptomics, in-situ-labelling proteomics, and mass spectrometry imaging (MSI) for spatial metabolomics, provide comprehensive spatial information within tissues. These approaches can elucidate cell-to-cell interactions, functional heterogeneity, and tissue complexity. While single-cell spatial transcriptomics and label-based proteomics have been applied to plant tissues like callus, leaves, and roots, high-resolution MSI still faces challenges due to tissue fragmentation and metabolite leakage during sectioning, especially in high-moisture tissues.

In our project, we explored suitable cryo-sectioning methods and developed a plant-adapted MSI method achieving cell-level resolution (10 µm). Using this method, we identified three main metabolic patterns in vertical leaf sections: epidermis, mesophyll, and main vein surroundings. Through metabolite identification, we localized various metabolites, including lipids and other functional compounds, in distinct regions. Additionally, we applied this method to the leaves of the Arabidopsis mutant PRODUCTION OF ANTHOCYANIN PIGMENT 1-DOMINANT (PAP1-D), successfully visualizing anthocyanins and their biosynthetic precursors. This work demonstrated the feasibility and effectiveness of this method.

In summary, we developed a high-resolution MSI method tailored to plant tissues. This advancement significantly enhances the understanding of plant spatial metabolomics, paving the way for the application of spatial multi-omics in plant research.

Identification of pesticidal PK/PBAN analogs for *Riptortus pedestris*

Ka Yin LAM and Chi Ki NGO

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Speaker

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The attribution of the piercing-sucking feeding behaviors of the soybean pest, the bean bug (*Riptortus pedestris)*, to the culprit of Soybean Staygreen Syndrome and the immense economic loss generated has long been discussed. Due to the ineffectiveness and non-specificity of the commercially available pesticides against the pest, the omnipresent insect neuropeptides emerge as an opportunity for the development of target-specific and bio-degradable pest control strategy, given their capabilities to orchestrate divergent physiological processes upon the binding to the G protein-coupled receptors (GPCR). Previous studies have unveiled the biological functions of the pyrokinin/pheromone biosynthesis activating neuropeptides (PK/PBAN) family members in different insect species. More importantly, pesticidal effects had been observed for certain stabilized PK/PBAN analogs. To date, the functional roles of PK/PBAN peptides in *R. pedestris* remain enigmatic. We are prompted to decipher the functions of PK/PBAN peptides in *R. pedestris*, hoping to utilize them as an efficacious tactic for pest management. Herein, three short PK/PBAN peptides (SB1-SB3) for *R. pedestris* were designed strategically and two corresponding PK/PBAN GPCRs (*Rp-PK/PBANR-1 and Rp-PK/PBANR-2)* candidates had been identified. Using Reverse Transcription Polymerase Chain Reaction (RT-PCR), we revealed a downregulation of *rp-pk/pbanr-1* and an upregulation of *rp-pk/pbanr-2* in the salivary glands of the female adult *R. pedestris* after 2-day intake of SB2 and SB3, respectively. This confirmed the peptides possess activity *in vivo.* Meanwhile, our MTT assay results showed that the peptides do not exhibit cytotoxic effects in the mammalian HEK293 cells, suggesting that they may be safe to be used as pesticides. The testing of SB2 and SB3 on insect behavioral changes are currently underway. We believe that our findings offer useful insight for designing stable peptide analogs and pave the experimental groundwork for continual study on the insect neuropeptides.

Structure of UreE and UreG Reveals How Urease Accessory Proteins Facilitate Maturation of Urease in *Helicobacter pylori.*

Chun Long CHAN and Kam Bo WONG

Speaker

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State Key Laboratory of Agrobiotechnology (CUHK) and

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Helicobacter pylori (H. pylori) colonizes the acidic stomach environment by relying on urease activation to produce ammonia and neutralize gastric acid, in which two nickel ions were required for the urease active site. Following the Irving–Williams series, nickel ions would replace the less competitive ions in metallochaperones and inactivate the metallochaperones. Thus, the free nickel ions are cytotoxic to a cell. H. pylori has evolved an elegant hydrogenase-urease maturation pathway, assisted by the other accessory proteins (HypA, UreE, UreG, UreF, UreD) to regulate the free nickel ions. The hydrogenase-urease maturation pathway described the nickel ions transferred from HypA → UreE → UreG → UreF/UreD → Urease by specific protein-protein interaction, yet the mechanism of nickel ion transfer from UreE to UreG remains unclear. To study the interaction between UreE and UreG, we have solved the crystal structures of the nickel-bound UreE2G2 complex. Structural insights will be tested by mutagenesis and functional assay. We have identified several conserved residues that could be important in the formation of the UreE2G2 complex and for nickel delivery in urease maturation. We will create variants of UreE and UreG and test their roles in UreE-UreG interaction and in urease maturation. How UreE receives its upstream nickel ions remains not fully understood as well. We have already identified the C-terminal of UreE could be important to receive its upstream nickel ions while the C-terminal of UreE has been proven to be not essential to the UreE-UreG interaction. We will test the interaction between UreE and HypA with or without the C-terminal of UreE. To address if nickel ions bound to the Ni-His complexes and His-rich motifs could provide the nickel source to UreE for urease maturation. We will also test the interaction between UreE and His-Ni with or without the C-terminal of UreE.

Houttuynia cordata Thunb. Extracts Alleviate Atherosclerosis and Modulate Gut Microbiota in Male Hypercholesterolemic Hamsters

Yuhong LIN and Wing Tak Jack WONG

State Key Laboratory of Agrobiotechnology (CUHK) and
School of Life Sciences, The Chinese University of Hong Kong

Speaker

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Hypercholesterolemia leads to cardiovascular diseases and atherosclerosis. Previous studies have highlighted the crucial role of gut microbiota in alleviating atherosclerosis progression and reducing plasma cholesterol. However, the protective effects of *Houttuynia cordata* Thunb (HCT), a well-known fishy Chinese herb, against hypercholesterolemia and vasculopathy remain largely unknown. This study aims to explore the effects of HCT extracts on vascular health and gut microbiota in golden Syrian hamsters with hypercholesterolemia. The hypercholesterolemia hamster model was established by feeding with a high-cholesterol diet. Aqueous or ethanolic HCT extracts were mixed with diet and concurrently given to hamsters for six weeks. Plasma lipid profiles were evaluated. Aortas were collected to detect fatty streak areas. Feces were collected to analyze the abundance of microorganisms in the gut microbiota. HCT ethanolic extract treatment remarkedly decreased plasma levels of total cholesterol and high-density lipoprotein cholesterol in hypercholesterolemic hamsters. Notably, both aqueous and ethanolic extracts of HCT reduced atherosclerotic plaques in hamsters fed with a high-cholesterol diet. Strikingly, the effects of HCT ethanolic extract in reducing atherosclerotic plaques are greater than aqueous extract. Furthermore, at the phylum level, the relative abundance of *Firmicutes* was decreased in hamsters treated with aqueous and ethanolic extracts of HCT. By contrast, the abundance of *Bacteroidetes* was increased by HCT treatment. At the family level, HCT extract favourably modulated the relative abundance of *Porphyromonadaceae* and *Bacteroidales_S24-7_group*. These findings indicate that HCT extracts may facilitate the growth of short-chain fatty acids-producing bacteria to alter gut microbiota composition, contributing to reducing plasma lipid levels. This study offers evidence demonstrating the effects of HCT extracts on alleviating atherosclerosis and lowering plasma cholesterol levels in the male hypercholesterolemic hamster model, offering novel insights into the pharmacological effects and promoting the application of HCT. This study highlights the potential of HCT as a dietary supplement to alleviate atherosclerosis, lower plasma cholesterol, and modulate the abundance of microorganisms in gut microbiota.

Insect Metamorphosis Regulated Differently between Sexes by Members of a microRNA Cluster

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Speaker 18

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The life cycle of *Drosophila* consists of four major stages: the embryo, larvae, pupae, and adult. The the sesquiterpenoid and the ecdysteroid hormone groups regulate the development and metamorphosis in many arthropods including insects. The two hormonal systems act antagonistically. The sesquiterpenoid hormones retain the juvenile features and the ecdysteroid hormones lead to molting and metamorphosis. The hormones regulate such developmental processes by down- and up-regulating certain transcription factor genes, reaching the down-stream genes expression alteration, and eventually initiate the metamorphosis processes in fruit flies. Recent research highlights the role of non-coding RNAs, particularly miRNAs, in this regulatory landscape.

Using the total RNA and small RNA sequencing of 3rd instar larvae and pre-pupae male and female of *w*^{1118} *Drosophila melanogaster* total RNA, we identify the differentially expressed genes (DEGs) and miRNAs (DE miRNAs) in this initiation of metamorphosis. KEGG pathway analysis revealed enriched pathways associated with these DEGs. *In silico* miRNA target predictions by TargetScanFly suggested the potential interaction between the miRNAs and mRNAs. The potential interaction of miRNAs and mRNAs were validated through dual-luciferase reporter assay. The down-regulated mir-277 and mir-34 cluster in male and female *Drosophila* metamorphosis were predicted to regulate the expression level of the up-stream and down-stream genes of the two hormonal pathways. Mutant miRNA knockout flies were created to further examine their characteristics. The transcriptome analysis suggests the mutants when compared to the wild type, have enriched specific pathways distinctively and collaboratively in male and female. The 3rd instar larvae and pre-pupae male and female wild-type and mutant flies were measured for hormonal titres and the hormone titres vary in sexes and life stages, indicating the two miRNAs regulate the hormonal and developmental pathways differently in life stages and sexes in insect metamorphosis.

Diurnal rhythm regulation on BCM1-mediated chlorophyll metabolism

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Speaker

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Chlorophyll (Chl) is essential for photosynthesis. Both a deficiency and an excess of Chl production can be detrimental to plant yield and fitness because of reduced light energy absorption and generation of reactive oxygen species (ROS). Chl metabolism is governed by the balance between Chl biosynthesis and degradation, which maintains a dynamic equilibrium of Chl levels within the plant. Our research has uncovered that the scaffold protein BALANCE of CHLOROPHYLL METABOLISM 1 (BCM1) interacts with GENOMES UNCOUPLED 4 (GUN4) to stimulate Mg-chelatase activity, a key step in Chl biosynthesis, and also promotes the proteolysis of STAY-GREEN 1 (SGR1). These functions are dependent on the C-terminal domain of BCM1, GUN4, and SGR1. However, the mechanism by which BCM1 alternates its interaction with GUN4/SGR1 and the regulation of the BCM1-mediated Chl metabolism pathway by plants are not yet fully understood. In this study, we demonstrate that Chl metabolism is influenced by diurnal rhythms, and this regulation is closely correlated with the competitive interaction between SGR1/GUN4 and BCM1. Notably, the crucial polar amino acids within the CT domains of BCM1, GUN4, and SGR1 were examined *in vitro* and *in vivo*. Collectively, these findings suggest that the steady-state of BCM1-mediated Chl metabolism, modulated by diurnal rhythms, is a consequence of competitive protein interactions at the posttranslational level, stemming from polar amino acids at the C-terminus of the committed enzymes in Chl metabolic pathway.