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IPMBAB

Abstract Booklet

Characterization of soybean acyl-CoA-binding proteins

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Speaker

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Acyl-CoA-binding proteins (ACBPs), found in all eukaryotes and some prokaryotes, contain a highly-conserved domain of around 90 residues that can bind to acyl-CoA esters, which are the essential intermediates in lipid metabolism. Investigations on non-leguminous plants such as *Arabidopsis thaliana* (thale cress) and *Oryza sativa* (rice), have revealed the importance of ACBPs in developmental and stress responses. Plant ACBPs have been classified into four classes according to their size and adjoining additional domains. Soybean (*Glycine max*) ACBPs, designated GmACBPs, are not well reported although this legume is a globally important crop cultivated mainly for its high oil and protein content. It also plays a significant role in the food and chemical industries. In this study, *in silico* analysis of GmACBPs identified 11 members grouped into four classes: two members in each of Class I (small) and Class II (ankyrin repeats), four members in Class III (large) and three members in Class IV (kelch motif). Their domain architecture was predicted and compared to Arabidopsis and rice ACBPs. Prediction of their tertiary structure and subcellular localization was determined. Data mining of RNA-sequencing analyses indicated individual *GmACBP* putative expression in various organs. The expression profile revealed high expression of some Class III *GmACBPs* in root nodules suggesting their involvement during nodulation, a role not previously encountered for the non-leguminous ACBPs. Hence, efforts are being made on unravelling the role of GmACBPs in nodulation.

Brassinosteroid signalling and regulation in soybean

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Speaker

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Brassinosteroids (BRs) as the sixth group of plant hormones, play significant roles in plant growth, development, and adaptation to the environment. Due to its vital importance, great progress has been made in the past few decades in understanding the mechanism of BR actions in the model plant *Arabidopsis thaliana*. To regulate the expression of various BR-responsive genes, BR signals are perceived by the plasma membrane receptor BRI1 and co-receptor BAK1, transduce through the positive regulators (BSK1, CDG1, BSU1 and PP2A) and the negative regulators (BKI1, BIN2, 14-3-3s), finally arrive the nucleus to control the activities of BES1 and BZR1, two core transcription factors in the BR signaling pathway. Despite these significant advances in BR signaling studies in *Arabidopsis,* the BR signaling pathway is poorly understood in soybean. The demand for enhancement of soybean yield is increasing in recent years has prompted us to study the potential application of BRs in soybean breeding and genetic improvement. Therefore, dissecting the BR signaling pathway in soybean is of great importance for grain production and agriculture.

The cultivated soybean (*Glycine max*) that has been widely planted come from the wild soybean (*Glycine soja*) through long-term domestication and improvement. Compared to cultivated soybean, wild soybean has the characteristics of multiple flowers and multiple pods, strong stress tolerance and adaptations to harsh environments, which is an important genetic resource of the soybean. In my thesis project, I will first study the different responses of cultivated soybean (W82) and wild soybean (W05) to BR treatment. BR effects on the growth of different soybean organs such as stem and root will be examined. After that, BR-induced global gene expression changes will be analyzed in the two germplasms by transcriptome profiling. Finally, the functions of selected key BR signaling components in soybean will be studied in both soybean and Arabidopsis to investigate their roles in plant growth and stress resistance.

Possible roles of a plant ribosome-associated protein on translational regulation

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Speaker

3

Plants face versatile environmental conditions. Regulated turnover of proteins helps to cope with the ever-changing environment. OsYchF1, an unconventional G-protein, is involved in modulating defense response and abiotic stress tolerance in rice. However, the molecular mechanism of YchF1 on stress response regulation remains unclear.

Ribosomal rRNA, ribosomal proteins, and 26S proteasome subunit are some of the known YchF1 interacting partners identified by our laboratory previously. We hypothesized that YchF1 might act as the stress regulator that regulates the turnover of the protein by translational activities on ribosomes and degradation on 26S proteasome. In particular, plant ribosomal proteins usually exhibit heterogeneity as multiple ribosomal protein-encoding genes were found in their genome. The association of YchF1 protein on the ribosome might change its composition, and alter specificity and affinity towards specific groups of mRNA as its bacteria, yeast, and human homologs do.

Our unpublished data showed that the YchF1 protein level might alter protein profiles in plants, which includes ribosomal proteins. It is postulated that the specific interaction between YchF1 and ribosome might shape the plant stress responses through translational regulation. To study the effect of YchF1 proteins on the translational landscape, translatomic analysis would be done by polysome profiling; and corresponding protein profiles would be identified by proteomics approaches.

In this study, the homolog of OsYchF1 in *Arabidopsis thaliana* (AtYchF1) is chosen as the model due to their sequence similarity, and its well-established genetic system. *AtYchF1* knockdown mutant and *AtYchF1* overexpressor will be used as the genetic materials to study the dose-dependent effect of the YchF1 level on the translation profile. A mutant form of AtYchF1, with the abandoned ribosomal protein RPS7 interacting ability, will be used to study the effect of ribosomal protein RPS7 binding on AtYchF1 function. Moreover, *Arabidopsis* seedlings will be subjected to high salinity to induce changes in translational profiles under abiotic conditions. This project aims to delineate the role of YchF1 and clues on the putative translational regulation in plants.

Bioenergetics of pollen tube growth in *Arabidopsis thaliana* revealed by ratiometric genetically encoded biosensors

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Speaker

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Pollen is the fastest-growing cell in plants, of which its elongation process requires an efficient energy supply and rapid membrane biosynthesis. New pollen membrane is synthesized in ER, while the production of fatty acids occurs in the pollen plastid. Unlike chloroplasts, pollen plastids do not undergo photosynthesis, and the bioenergetics in pollen tubes remain largely unknown. To clarify the energy conversion processes in growing pollen tubes, we developed genetically encoded ratiometric fluorescence sensors for pyridine nucleotides which are pH insensitive between pH 7.0 to pH 8.5. The biosensors for measuring dynamic changes of ATP, NADPH, and NADH/NAD^+ ratio were introduced to the cytosol and plastids of Arabidopsis pollens under the control of a pollen-specific Lat52 promoter. By transforming these biosensors into the various mutants of key metabolic enzymes or by treating the sensor lines with specific inhibitors of different biochemical pathways, we depict the energy metabolism within elongating Arabidopsis pollen tubes, especially in pollen plastids. We also show that fermentation and pyruvate dehydrogenase bypass are not essential for pollen tube growth in Arabidopsis, in contrast to the other plant species like tobacco and lily.

An assessment of the crop production losses caused by ambient ozone in China from 2005 to 2019 using both concentration-based and flux-based metrics

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Speaker

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Exposure to elevated surface ozone can cause substantial crop production reductions worldwide, which poses a serious threat to global food security, especially in developing Asian countries. In this study, modelled hourly ozone concentrations from GEOS-Chem are corrected by machine learning approach to study the long-term ozone-induced crop production losses (CPLs) for key crops in China from 2005-2019. Here we provide a comprehensive analysis and comparison of the estimated CPLs using three concentration-based (AOT40, M7/M12 and W126) and two flux-based metrics (POD_3_FBB and POD_3_DO_3SE). Our results show that larger discrepancy in CPLs estimates comes from methodological differences compared to ozone concentration variability. AOT40 using Chines-specific exposure-response functions always give higher estimated CPLs than other metrics and functions developed in US and Europe, while flux-based metrics always give the lower losses. The 15-year average annual RYLs for maize, winter wheat, soybean and rice range between 1.1–7.8%, 1.0–30.2%, 4.0–25.5%, and 2.1–19.3%, respectively (range resulting from different metrics used). Pooling all metrics together, the annual national mean RYLs (CPLs) for wheat, rice, maize, and soybean ranged 7.1%–9.8% (4.8–9.8 Mt), 5.6%–7.8% (5.3–9.1 Mt), 2.5%–4.5% (3.8–15.4 Mt) and 7.0%–12.0% (0.6–1.4 Mt) from 2005 to 2019, accounting for annual average economic loss of 2.4 billion USD yr^{-1}, 3.2 billion USD yr^{-1}, 3.9 billion USD yr^{-1}, and 0.7 billion USD yr^{-1}. On a province scale, the averaged total production loss for all crops is greatest in Henan (3.65 Mt yr^{-1}), Shandong (3.22 Mt yr^{-1}) and Hebei (3.18 Mt yr^{-1}). To our knowledge, this study provides the first long-term estimation of CPLs using multiple concentration-based and flux-based metrics. Although uncertainties remain, our findings show that ozone pollution have caused substantial crop production loss in China from 2005 to 2019, resulting in great economic losses. This study could provide an important reference of the uncertainty in CPLs estimates in China induced by methodological differences, which facilitates the intercomparison of subsequent studies using different methods.

Mapping foliar photosynthetic capacity in sub-tropical and tropical forests with UAS-based imaging spectroscopy: scaling from leaf to canopy

Shuwen LIU and Jin WU

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Speaker

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Accurate understanding of the variability in foliar physiological traits across landscapes is critical to enable improved parameterization and evaluation of terrestrial biosphere models (TBMs) that seek to represent the response of terrestrial ecosystems to a changing climate. Increasing studies suggest the great potential of imaging spectroscopy for characterizing foliar biochemical and morphological traits at the canopy scale, but it remains unclear whether it could help infer foliar photosynthetic capacity (e.g., maximum carboxylation rate, $V_{c,max}$ and maximum electron transport rate, J_{max}). To advance the spectra-trait approach and enable the estimation of key traits using remote sensing, we collected Unoccupied Aerial System (UAS) data over two forest sites in China (a subtropical forest in Mt. Dinghu and a tropical rainforest in Xishuangbanna), where UAS-based canopy spectra (93 bands covering 502-870 nm) were acquired, together with ground measurements of leaf spectra and biochemical (leaf nitrogen, phosphorus, chlorophyll, and water content), morphological (leaf mass per area, LMA) and physiological ($V_{c,max25}$ and J_{max25}) traits (n=135 tree-crowns from 42 species across two sites). We used the partial least-squares regression (PLSR) to build spectra-trait models and evaluated them with repeated cross-validation. The spectral models developed using leaf spectra were transferred to canopy spectra to assess the effect of canopy structure. We used spectral models to map these traits at individual tree-crown scale and analyzed their inter- and intraspecific variance. The results shows that (1) UAS-based canopy spectra could be used to estimate $V_{c,max}$ (R^2=0.55, RMSE=8.48 µmol CO_2 m^{-2} s^{-1}), J_{max} (R^2=0.54, RMSE=16.78 µmol CO_2 m^{-2} s^{-1}), and five additional foliar traits (R^2=0.38-0.60) at the tree-crown scale with demonstrated generalizability across two sites; (2) UAS-based imaging spectroscopy maps large variability in all foliar traits (including physiological traits) with spatially explicit information, covering the inter- and intra-specific variations. These results demonstrate the capability of using UAS-based imaging spectroscopy for characterizing the variability of foliar physiological traits at individual tree-crown scale over forest landscapes and highlight the similar generalizability but different biophysical mechanisms underlying spectra-trait relationships at leaf and canopy levels.

Transcriptional regulation of the Casparian strip formation in maize root exodermis

Weilun LIU and Silin ZHONG

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Speaker

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Root architecture is optimized to selectively absorb nutrients and water, while protecting against toxic compounds and pathogens. Selectivity of the root 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 similar as endodermis. 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 enabled the identification of endodermis and exodermis cell clusters and the genes specifically expressed in them. We also conducted single cell ATAC-seq to identify differential accessible chromatin regions (ACRs) in endoermis and exodermis cell clusters, along with enriched TF binding motifs in ACRs. All these showed that different sets of CASPs were expressed in endodermis and exodermis suggesting that the two types of cells utilize different sets of genes to construct CSs. In addition, gene regulatory inference for TFs enriched in exodermis analysis using the scRNA-seq data predicted several TFs to regulated genes invloved in CSs formation. Future work will utilize techniques such as ChIP-seq and dual-luciferase reporter assay to validate these regulatory interactions. This work would provide to a deep understanding on the transcriptional controls of CSs formation in maize root exodermis.

Transcriptional Regulation of Vacuole Biogenesis in Plants

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Speaker

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Vacuole is one of the important membrane-bound organelles in the plant endomembrane system that plays important roles in plant growth and development (Cui *et al*., 2016). Multiple transport pathways and regulators have been shown to mediate vacuolar transportation while multiple models of vacuole formation have been proposed in plants, including the recent model of multivesicular body (MVB)-MVB fusion leading to the vacuole formation of small vacuoles in *Arabidopsis* root cells, which was based on whole-cell electron tomography (ET) analysis (Cui *et al*., 2019).

Studies in mammalian cells have illustrated transcriptional regulation of lysosome biogenesis (Sardiello *et al*., 2009; Palmieri *et al*., 2011; Martina *et al*., 2014). However, little is known about transcriptional regulation of vacuole formation in plants. Here we test the hypothesis that vacuole formation in plants is also regulated by specific transcription factors (TFs). Towards this goal, we have first screened and identified TF candidates that regulate vacuole biogenesis in *Arabidopsis thaliana* through two approaches: 1) Bioinformatic analysis of vacuole-related genes to generate the upstream TF candidates; and 2) Yeast-one hybrid screening to identify TFs for regulating key vacuole-related genes. Next, *Arabidopsis* T-DNA insertional mutants and overexpression lines of the newly identified TF candidates have also been generated for subsequent analysis on vacuole phenotype in root cells vs. the wild type plant. So far, we have identified several mutants of TF candidates affecting normal vacuole formation, including the mutant of *AUXIN RESPONSE FACTOR 5* (*ARF5*), a TF gene of B3 family protein, which exhibited tubular vacuolar phenotype in mutant root cells. We have further tested and confirmed the expression levels of vacuole-related genes under the regulation of ARF5 by both dual luciferase assay (Dual-LUC) and quantitative RT-PCR assay (qRT-PCR). Future studies such as Chromatin Immunoprecipitation (ChIP) and Electrophoretic Mobility Shift Assay (EMSA) will be performed to verify the interaction between ARF5 and the target gene promoters. Taken together, our preliminary data support our hypothesis while further identification and characterization of TF candidates in regulating vacuole formation will facilitate understanding about their downstream targets and underlying mechanisms in plants.

Functional characterization of *Arabidopsis* voltage-dependent anion channels (VDACs) in mitophagy

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Speaker

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The mitochondrion is an essential organelle in eukaryotes mediating cellular respiration and intracellular signaling pathways. To maintain a healthy mitochondrial population, dysfunctional and surplus mitochondria will be removed via a type of selective autophagy named mitophagy. In the last decades, a series of autophagy-related (ATG) proteins have been characterized, and they are required for mitophagy in yeast and mammalian cells. Although the core autophagy machinery that mediates autophagosome formation is highly conserved in plants, the mechanism of mitophagy in plant cells is still poorly understood.

Recently, our lab demonstrated that depolarized mitochondria induced by uncouplers (DNP and FCCP) are recycled by mitophagy in *Arabidopsis*. In fluorescent and electron micrographs of uncoupler-treated cells, we observed aberrant mitochondria selectively enclosed by mitophagosomes. Nevertheless, molecular mechanisms as to how depolarized mitochondria are recognized and captured by mitophagosomes remain elusive.

We employed YFP-fused ATG8 as an autophagy marker to identify the key proteins in the plant mitophagy process via immunoprecipitation-mass spectrum (IP-MS) assay. Voltage-dependent anion channels (VDACs), one of the most abundant proteins in the outer mitochondrial membrane, were enriched in the ATG8-interactome after DNP treatment. The *Arabidopsis* genome encodes several VDAC paralogs and some of them co-immunoprecipitated with YFP-ATG8e after mitochondria depolarization. Protein-protein interaction assays indicated that VDACs interacted with ATG8 directly. Confocal microscopy and transmission electron microscopy (TEM) images showed that damaged mitochondria accumulated in *Arabidopsis* root cells, and mitophagy was affected in mutants of those VDAC paralogs. These data provide evidence that VDACs are involved in the autophagic recycling of damaged mitochondria in Arabidopsis.

It has been demonstrated that VDACs are modified by other regulators in eukaryotic cells under stress conditions. For example, *DmVDAC* is phosphorylated by GSK3 in ubiquitin-proteasome system (UPS) activity. In mammalian cells, monoubiquitination of VDAC prevents apoptosis, while polyubiquitination contributes to mitophagy. Moreover, VDACs oligomerize in oxidatively stressed mitochondria to release short mtDNA fragments. We are currently investigating whether VDAC modifications are associated with plant mitophagy.

Functional study of the transcription factor Yin Yang1 in the mouse cerebellar Purkinje cells

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Yin Yang 1 (YY1), a zinc-finger transcription factor, has a dual nature in regulating gene expression due to its inhibitory and activation domains. YY1 has diverse roles in mammalian development and growth, including regulation of cell proliferation, apoptosis, and differentiation. YY1 achieves such functions based on its ability to disrupt DNA binding transcription factors, recruit transcription co-factors, and modify DNA conformation. Our preliminary data have shown that inactivation of Yy1 in the cerebellum would lead to abnormal postnatal Purkinje cell (PC) dendritogenesis, which impairs cerebellar function and results in motor coordination defect, severe ataxia phenotype and poor embryonic brain development. As PCs serve as an integration center in cerebellar circuits, it is essential to understand the association of YY1 and its potential ability in intrinsic control of the PC dendrite growth in order to gain insights into genetically inherited motor disability diseases in humans.

Our work utilized the ataxic Yy1 conditional knockout mouse model (*Yy1*-cKO) to investigate the role of Yy1 in postnatal mice PC development, with a major focus on dendritogenesis. *Yy1*-cKO postnatal mutant mice PCs showed smaller cell soma and poor dendritogenesis, which resulted in shorter molecular layer thickness, as well as less synaptic vesicles in the parallel fiber-PC synaptic innervation. Our results suggest silencing *Yy1* in PCs could cause defects in synaptogenesis with protein kinase C (PKC) signaling mediation.

Based on *in silico* transcriptomic analysis, we hypothesized the downregulation of *Wnt10b*, a potential Yy1 downstream gene, could regulate PC dendrite development. The hypothesis was then validated with *in vivo* transcriptomics, in which *Wnt10b* was downregulated. Few studies have suggested Wnt/β-catenin signaling, which Wnt10b belongs in, regulates axon regeneration in injured CNS and various developmental processes during embryogenesis, cancer, and hair follicle development. However, the roles of *Wnt10b* in cerebellar development remain elusive.

To further investigate the role of Yy1-*Wnt10b* interaction in dendrite development, we utilized a *Wnt10b*-knockdown (Wnt10b-KD) model in organotypic cerebellar slice culture. Our results showed that Wnt10b knockdown resulted in less complex dendrite development. Yy1 could interact with the *Wnt10b* promoter sequence *in vivo* by Chip-PCR assay. Moreover, our results showed that the PC of Wnt10b-KD cerebellar slices showed similar phenotypes to those in Yy1-cKO mice.

Based on our current results, we suggest that the postnatal PC development regulated by Yy1 could be mediated by its interaction with *Wnt10b* and the Wnt/β-catenin signaling.

Structural basis of pH-dependent chaperone function of a small heat shock protein

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Mycobacterium tuberculosis (Mtb) is the pathogen of Tuberculosis (Tb), one of the most killing infectious diseases worldwide. Challenged by the developing Multi-Drug-Resistance Tb, a new therapy is demanded. Small heat shock protein 16.3 (Hsp16.3) has been identified as chaperone in Mtb which associates with early proteins misfolding. Recently it attracted attention as a drug target for Tb since it was found indispensable for the survival, persistence and virulence of Mtb. Despite its great therapeutic potential , the drug development was hindered by a lack of structure. Also interesting is that Mtb can resist acid in macrophages by entering dormancy, a status which Mtb shuts down most metabolic while highly expresses Hsp16.3. Therefore, we demonstrated it behaves differently at different pH and resolved its structures under different pH conditions to around 3-4 Å using cryo-EM, a technique that is ideally suited for structural studies of highly complex and heterogeneous samples.

Role of sarcoplasmic reticulum and mitochondria communication in the maturation of embryonic stem cell-derived cardiomyocytes

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Speaker

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Embryonic stem cell-derived cardiomyocytes (ESC-CMs) have potential benefits of cell-based therapy, disease modeling, and drug toxicity testing. However, under the conventional differentiation protocol of ESC-CMs, ESC-CMs are still immature, which hampers their further clinical applications. Immature ESC-CMs and adult cardiomyocytes differ in metabolism, electrophysiological characteristics, Ca^{2+} handling and cellular morphology. Previous studies suggested that mitochondria play a critical role in shaping metabolism during maturation of cardiomyocytes; it has also been known that mitochondria shape the cytosolic Ca^{2+} of cardiomyocytes. On the other hand, sarcoplasmic reticulum (SR) is known to communicate with mitochondria for Ca^{2+} signaling, lipid exchange, etc. In this study, we hypothesize that SR-mitochondria communication is required for regulating mitochondrial and cytosolic Ca^{2+}, mitochondrial biogenesis, and metabolism, and these would eventually direct ESC-CMs to a more mature status.

To test our hypothesis, mitofusin 2 (MFN2), a protein known to regulate the tethering of SR and mitochondria, was knocked down in ESC-CMs. Knockdown of MFN2 decreased the overlapping of SR and mitochondria and decreased the mitochondrial Ca^{2+} increase induced by caffeine, suggesting a decrease in SR-mitochondria communication. Interestingly, ESC-CMs with MFN2 knocked down displayed less mitochondrial area and mtDNA, suggesting a decrease in mitochondrial biogenesis. Consistently, knockdown of MFN2 decreased the expression of mitochondrial transcription factor A (TFAM), which is coded in nucleus and regulates mtDNA expression in mitochondria. Importantly, knockdown of MFN2 decreased cell size, led to a less organized sarcomere structure, reduced expression of genes related to fatty acid oxidation, and decreased cytosolic and mitochondrial Ca^{2+} in ESC-CMs. The results suggested that MFN2 positively regulates the maturation of ESC-CMs. Next, we will evaluate action potential and metabolic status to further confirm the role of MFN2 in the maturation of ESC-CMs. In addition, the mechanisms of how SR-mitochondrial communication regulates the level of TFAM will also be elucidated.

Our study will provide new insights into whether SR-mitochondrial communications can contribute to the maturation of ESC-CMs and potentially suggest that manipulation of SR-mitochondrial tethering would be a way to promote the maturation of ESC-CMs.

Genomics and biology of RNA G-quadruplexes in the malaria parasite *Plasmodium falciparum*

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Speaker

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Human malaria is caused by protozoan parasites of the *Plasmodium* group. Most of the deaths from the disease are caused by the species *P. falciparum*, which has an unusual genome with an extreme A/T-bias of ~81%. RNA G-quadruplexes (rG4s) are important secondary structures that can form in guanine-rich RNA sequences. These four-stranded motifs have been demonstrated to play significant regulatory roles. Despite the scarcity of guanine-rich sequences, rG4s has been predicted in some *P. falciparum* gene regions, notably in the CDS and UTR of the major virulence gene family *var/PfEMP1.*

To explore the biology of rG4 in this parasite, we performed a transcriptome-wide rG4 profiling using rG4-seq and rG4-seeker. The experiment identified more than 2500 rG4s from ~1300 genes, where the majority were found in the CDS region. These rG4s motifs were shown to be non-randomly distributed in the transcriptome – they were concentrated at local regions with a spike in G/C content, which was driven by nucleotide repeats and/or peptide low complexity regions. A 3-base periodicity of G/C ratio fluctuations was also observed in rG4s and their flanking sequences, suggesting the integration of rG4 motifs into codon sequences. In addition, our analysis also revealed a modest enrichment of rG4-harboring genes in pathogenicity and transcriptional regulation pathways.

The other significant finding of the study was that almost all *P. falciparum* rG4s identified were non-canonical (NC) rG4s. Interestingly, unlike their canonical counterparts, most of these NC rG4s have not been previously predicted. Since NC rG4s could form the quadruplex structure using fewer and less densely packed guanine residues, they also seem better adapted to the A/T-biased genome. Our study further showcased that NC rG4s could equivalently reduce protein translation efficiency *in vivo* by stalling ribosomes. Moreover, the observations in *P. falciparum* agreed with our prior findings in humans, where NC rG4 was found to be the dominant species of rG4s in both transcriptomes.

Together, our study suggested that rG4s are an integral feature of *P. falciparum* transcriptome and potential therapeutic targets for malaria disease. Moreover, the detailed landscape of rG4s in this early-diverged eukaryote provided insight into the evolution of rG4s.

Understanding protein evolution in C4 photosynthesis: co-evolution, transcriptome signature, and similarities divergence

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Speaker

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C4 photosynthesis is crucial for plants that live in arid and thermal habitats and demonstrate a decent example of convergence evolution among over 60 species. As superior to the ancestral C3 type of photosynthesis, the distinctive anatomy of C4 photosynthesis and its two-celled carbon delivery biochemistry has been well illustrated. To engineer the C4 traits in C3 crops, knowing the evolutionary coupling events and C3-C4 divergence will benefit us to select the appropriate C4 gene donors to establish the stable C4 pathway in C3 crops.

Here we report the global co-evolution relationship between known eight C4 enzymes, and also the co-varying sites inside themselves. Our study also reveals the importance to investigate the function of the N-terminus of these enzymes in the C4 carbon delivery process. Furthermore, we examined the cell-specific transcriptomes of known C3 and C4 grass and identified twenty C3-C4 commonly shared signature genes. Among these genes, we found two flip-over genes (FOGs) that possess opposite cell-specific expression patterns in C3 and C4 model plants, and can be used to distinguish C4 grass from C3 ones. Moreover, we also verified the predictive performance of AlphaFold2 on plant protein structure prediction and utilized it to predict the structures of two FOGs in these model plants. And we found that based on current measurements of protein sequence and structure, the similarity score is inconsistent. And it is promising to come up with a new algorithm to overcome this flaw.

Our study may contribute to the selection of appropriate C4 gene donors in the engineering of C4 traits in C3 plants, by providing a structure-guided gene selection approach.

Conservation Genomics of the Incense Tree *Aquilaria sinensis* and its Associated Moth *Heortia vitessoides*

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The incense tree, *Aquilaria sinensis* (Lour.) Spreng. (Thymeleaceae), is one of the tree species that produces agarwood, formed by the impregnation of aromatic resins in the heartwood through antimicrobial defence mechanism. Due to the high commercial value of agarwood, incense trees in Hong Kong have long been under threats from illegal exploitation, impairing the local population and biodiversity. Although the Agriculture, Fisheries and Conservation Department has been actively planting seedlings of incense trees at the country parks of Hong Kong, the genetic diversity of the incense trees in Hong Kong is remained unknown and the lack of molecular information to prove the locality of illegally harvested agarwood has been hindering the process of gathering crime evidence.

With a view to conserve and investigate the genetic diversity of this valuable tree species, a population study of *A. sinensis* was conducted with 347 samples from mainland China (n = 40) and Hong Kong (n = 307). Using genome-wide single nucleotide polymorphisms (SNPs), population analyses revealed population clusters from Guangxi-Hainan in mainland and a “D7” region in Hong Kong. Genomic scan between population clusters further identified outliers for selection. Furthermore, genomic DNA of 7 natural agarwood samples were extracted and applied in next-generation sequencing, in which 2 sequenced agarwood samples were successfully mapped to the reference genome and clustered to the corresponding localities in phylogenetic tree analyses. These results could provide insights into the application of molecular approaches for the conservation and legal protection of the incense tree.

On the other hand, *Heortia vitessoides* Moore (Crambidae) is a specific moth species that depends on *A. sinensis* as a host to complete its life cycle, in which such plant-insect interaction may shed light on the conservation of the incense tree and its associated biodiversity. Therefore, comparative genomic analyses were conducted on a high-quality genome of *H. vitessoides* with other lepidopteran species. Rapid evolving genes related to detoxification and important metabolism pathways in *H. vitessoides* have been identified. Moreover, from the sequencing of small RNA of individuals at different life stages, 85 conserved and 96 novel micro RNAs were annotated, of which some appeared in forms of clusters. These current results could provide a genomic basis for further investigations in the plant-insect interaction between *A. sinensis* and *H. vitessoides*.

Asperuloside alleviates vascular dysfunction and atherosclerosis via activating endothelial Nrf2 signaling

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Speaker

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Atherosclerosis is characterized by endothelial dysfunction and plaque formation in the arterial wall. Unstable plaques are vulnerable to rupture followed by thrombotic occlusion and tissue infarction. This in turn can lead to ischaemic stroke or heart attack, threatening millions of people. It is urgent to develop effective treatment because current therapies are insufficient to successfully prevent atherosclerosis development and subsequent cardiovascular complications. Attenuating endothelial dysfunction is regarded as one of the therapeutic strategies for preventing vascular dysfunction and atherosclerotic plaques formation. Notably, the Nrf2 pathway is reported to be an essential pathway for clearing excessive reactive oxygen species (ROS) that mediates endothelial dysfunction. Thus, bioactive compounds targeting on Nrf2 activation can be promising candidates for treating atherosclerosis. In this proposed study, we intend to investigate the potential vascular protective effects of asperuloside (ASP), which is one of the major iridoids present in Eucommia species.

We hypothesize that ASP attenuates vascular dysfunction and atherosclerotic plaques formation through regulating Nrf2 pathway. In the endothelium, ASP activates Nrf2 activity and its downstream regulatory network to rescue endothelial dysfunction, the initial step of atherosclerosis, thus attenuating atherosclerosis progression.

Overexpression of IL-1β initially contributes to the progression of atherosclerosis. Our preliminary findings revealed that ASP significantly ameliorated IL-1β induced vascular dysfunction, showing the potential to attenuate atherosclerosis. Decreased Nrf2 signaling was found in both the atherosclerotic aorta and the aortic endothelial cells, indicate that activating Nrf2 signaling is essential for attenuating atherosclerosis progression. Moreover, our results showed that ASP rescued endothelial dysfunction through regulating Nrf2 activity, suggesting ASP is a Nrf2 activator. In conclusion, ASP is potential to prevent vascular dysfunction and attenuate atherosclerosis progression though regulating Nrf2 pathway in endothelium.

Arabidopsis DXO1 activates RNMT1 to methylate the mRNA guanosine cap

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Speaker

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Eukaryotic messenger RNA (mRNA) typically contains a methylated guanosine (m^7G) cap, which mediates major steps of mRNA metabolism. Recently, some RNAs in both prokaryotic and eukaryotic organisms have been found to carry a non-canonical cap such as the NAD cap. Here we report that Arabidopsis DXO family protein AtDXO1, which was previously known to be a decapping enzyme for NAD-capped RNAs (NAD-RNA), is an essential component for m^7G capping. AtDXO1 associates with and activates RNA guanosine-7 methyltransferase (AtRNMT1) to catalyze conversion of the guanosine cap to the m^7G cap. *AtRNMT1* is an essential gene. Partial loss-of-function mutations of *AtRNMT1* and knockout mutation of *AtDXO1* reduce m^7G-capped mRNA but increase G-capped mRNAs, leading to similar pleiotropic phenotypes, whereas overexpression of *AtRNMT1* partially restores the *atdxo1* phenotypes. This work reveals an important mechanism in m^7G capping in plants by which the NAD-RNA decapping enzyme AtDXO1 is required for efficient guanosine cap methylation.

Co-evolution between legumes and *Bradyrhizobium* from a time perspective

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Speaker

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The alphaproteobacterial genus *Bradyrhizobium* has evolved a remarkable ability to establish a mutualistic relationship with legumes by forming nodules and providing their host with N_2 fixed from the air. One of the most important questions is when this intimate host-symbiont interaction started. Timing the evolution of the nodulating ancestor of *B. japonicum*, however, is very difficult because of the lack of bacterial fossils and the underappreciated diversity of non-nodulating members of *Bradyrhizobium*. Here, we isolate 78 *B. japonicum* strains from diverse non-legume plants and have their genomes sequenced. Phylogenetics analysis showed that they formed three independent clades that diverged earlier than nodulating *B. japonicum*, suggesting that modern nodulating *B. japonicum* members derived from their non-nodulating relatives. We further adopt a recently developed approach based on mitochondrial endosymbiosis to co-estimate the evolutionary timelines of both *Bradyrhizobium* and their legume host. We show that the crown groups of legumes and nodulating *B. japonicum* both originated at around 100-150 million years ago, highlighting an important role of an increased diversity in the host plants in driving lifestyle diversification of *Bradyrhizobium*.

Examination of mercury content in local rice grains of different cultivars

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Speaker

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Recent studies have shown that rice can be a significant source of mercury exposure to humans, especially in inland areas where another main source of mercury, fish, is not available. In some polluted areas of China, rice consumption could account for 89-97% of mercury (Hg) exposure in local populations. In this study, locally grown rice grains of different cultivars from different farmers in Hong Kong have been analysed to determine their total mercury content. It was found that while the mercury content was lower than that of other studies, there is a noticeable difference in Hg content among cultivars, as it ranges from 1.2 to 2.7 ng/g among different cultivars of rice. One cultivar, Pai Hok Glutinous, has considerably more Hg (2.69 ng/g) than the others (1.39-1.94 ng/g on average). However, the Hg levels in all the rice grains are still much lower than those grown in polluted areas in mainland China, where the Hg level could be up to 500 ng/g, and our rice samples do not exceed the Chinese National Standard Hg limit (20 ng/g) for rice. The Hg level also fluctuates among different grains in the same cultivar (30-40% difference). More different rice cultivars from Hong Kong and Guangdong Province are under analysis, and data will be presented during the seminar. It is hoped that by finding out the variation of Hg content among cultivars, we can select the few cultivars with the highest Hg content and design further experiments to reduce its Hg accumulation in greenhouse experiments.