香港中文大學 CUHK
農業生物技術國家重點實驗室

SKLA / IPMBAB SEMINAR SERIES | 2019

IPMBAB

9 NOVEMEBER HONG KONG

Abstract Booklet

Brassinosteroid regulates chlorophyll homeostasis and chloroplast development in *Arabidopsis*

Wing-Shing WONG and Junxian HE

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

Chloroplasts are essential organelles which dedicate to carry out photosynthesis to provide energy for plant survival and development. Normal biogenesis and development of chloroplasts ensure plants to obtain optimal amount of energy from photosynthesis. Brassinosteroids (BRs) are steroidal hormones essential for normal plant growth and development. However, little is known to their roles in chloroplast development.

Therefore, in my MPhil thesis project, I studied the regulatory function of BR in chloroplast development in *Arabidopsis thaliana* by using physiological and cell biological approaches. From my observations, BR mutants display dark-green or light-green leaf colour depending on their different effects in BR synthesis or signaling. Physiologically, I revealed that BR could negatively regulate chlorophyll biosynthesis and positively regulate chlorophyll degradation to control the chlorophyll level in chloroplasts. The microscopic analyses to the cells and chloroplasts from different BR mutants using both light and transmission electron microscopy indicated that changes of BR levels or signaling in the mutant plants led to altered chloroplast number in given leaf area, changed mesophyll cell size and density, yet without affecting the core structure of chloroplasts.

In summary, my studies brought new insights and perspectives to the function of BRs in chloroplast development at the physiological and cell biological levels, which are valuable to future studies and better understanding of the mechanisms of chloroplast development.

Molecular characterization of FREE1 suppressors in *Arabidopsis thaliana*

Ying ZHU and Liwen JIANG

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

2

All eukaryotic cells contain several functionally distinct membrane-enclosed organelles in their endomembrane system, including the endoplasmic reticulum (ER), the Golgi apparatus, trans-Golgi network (TGN), endosomes, prevacuolar compartment (PVC) or multivesicular body (MVB) and vacuole/lysosome. The endosomal sorting complex required for transport (ESCRT) machinery mediates the formation of intraluminal vesicles (ILVs) in MVBs as well as the sorting of the ubiquitinated cargoes into the internal vesicles. We have recently identified and characterized a plant unique ESCRT key component FYVE domain protein required for endosomal sorting 1 (FREE1) with several novel findings. We demonstrated that FREE1 is essential for plant development, MVB ILVs biogenesis and vacuolar sorting of membrane proteins (Gao et al., 2014; Gao et al., 2015). To further illustrate the underlying mechanisms of FREE1 multiple functions in plants, a forward genetic screen was performed for mutants that suppressed the seedling lethal phenotype of *FREE1-RNAi* transgenic plants. The obtained mutants are termed *suppressors of free1* (*sof*) (Zhao et al., 2015). The first characterized *sof* mutant was *sof524*, which was identified as a *BRo1-domain protein As FREE1 suppressor* (*BRAF*) that negatively regulated MVB biogenesis and MVB-mediated protein sorting, thus representing a novel mechanism in plants (Shen et al., 2018). Another novel mutant *sof100* was identified and characterized. The corresponding gene *RESURRECTION1* (*RST1*) is shown to be a negative regulator mediating membrane protein homeostasis and FREE1-related functions in plants, representing a novel mechanism in regulating MVB biogenesis and trafficking in plants (Zhao et al., 2019). On-going study focuses on the characterization of two FREE1-related mutants, *sof10* and *sof641*, that have mutations in the same gene SOF10A. The *sof10* possesses a C-to-T mutation which results in a premature stop codon, while the G-to-A mutation in *sof641* produced a mis-sense mutation (Asp787-to-Asn). Both *sof10* and *sof641* seedlings exhibited reduced FREE1 protein level, normal vacuole and MVB morphology, as well as normal membrane protein degradation. Preliminary subcellular localization study showed that SOF10A exhibited cytosol pattern plus some punctate dots with unknown identity. Biochemical analysis suggested that SOF10A distributed in both cell soluble (CS) and cell membrane (CM) fractions. SOF10A also interacts with ESCRT-II component Vps36, suggesting that SOF10A may regulate the function of FREE1 through ESCRT machinery. SOF10A has a close homolog termed SOF10B. Further characterization of SOF10A and SOF10B using a combination of cellular and genetic approaches will illustrate the underlying mechanisms of SOF10 function as FREE1 suppressor as well as its roles in membrane protein trafficking and organelle biogenesis in plants. Here I will present an update on this study.

Photorespiration is the major source of NADH to mitochondria during photosynthesis

Shey-Li LIM and Boon Leong LIM

State Key Laboratory of Agrobiotechnology (CUHK)

School of Biological Sciences, University of Hong Kong

3

Mitochondria is the major ATP producer via mitochondrial electron transport chain (mETC). In mitochondrial matrix, NADH is oxidized by internal NDHs and Complex I, the first enzyme in mETC. The electrons from NADH are passed to oxygen through the mETC, which generates a proton gradient for the Complex V to synthesize ATP. In decades, there is an argument on the main source of NADH in mitochondria during the daytime. In some literature, NADH is depicted to be indirectly imported into mitochondria through the malate-oxaloacetate shuttle. Alternative, some literature reported that photorespiration is the main source of NADH in mitochondria. Here, we introduced the genetically encoded fluorescence pyridine nucleotides (NADPH and NADH/NAD^+) sensors into several Arabidopsis compartments namely, cytosol, chloroplast and peroxisome. By using in planta imaging, when cotyledon was treated with aminoacetonitrile (AAN), an inhibitor for glycine decarboxylate in the glycine-serine pathway in mitochondria, the ability of illumination in increasing the NADPH and the NADH/NAD^+ pool in plastid stroma was lost, while the synthesis of ATP in stroma and ETR were still operating. These results corroborate with the changes in the NADPH and NADH/NAD^+ level in cytosol, instead of being more oxidized under normal condition, the cytosolic compartment was relatively reduced under AAN treatment. These results indicate that under normal condition, a large amount of reducing equivalents is generated in mitochondria through photorespiration, which exceeds the NADH dissipating-capacity of mitochondria, and surplus reducing equivalents are therefore exported to the cytoplasm through the malate-oxaloacetate shuttle.

Establishment of C4 photosynthesis in the maize leaf involves enhanced regulation of solute transport in the bundle sheath cell

Peng GAO and Prof. Byung-Ho KANG

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

4

The chloroplast is a member of the plastid family responsible for photosynthesis. The thylakoid is a photosynthetic membrane in the chloroplast in which constituents of the electron transport chain are arranged.

Some plants growing in arid climate are capable of suppressing the oxygenation reaction of Rubisco by concentrating carbon dioxide (CO_2) around the enzyme. This mode of photosynthesis is called C4 photosynthesis, and it involves two types of chloroplasts. In the maize (*Zea mays*) leaf, the two types of chloroplasts are partitioned in bundle sheath cells (BSCs) outside the vascular bundle and mesophyll cells (MCs) surrounding BSCs, the functional differentiation of chloroplasts accompanies distinct arrangements of macromolecular complexes in their thylakoids. During maize photosynthesis, malate is produced in MCs from atmospheric CO_2 and transported to chloroplasts in BSCs where the Calvin Cycle operates using CO_2 released from malate. The differentiation of MCs and BSCs chloroplasts in maize is a critical event for establishing its C4 photosynthesis pathway.

Electron microscopy analyses of maize leaf cells have shown that thylakoids in MCs and BSCs are differentially organized. Thylakoids in MCs have grana stacks and interconnecting stroma thylakoids while thylakoids in BSCs consist mostly of unstacked planar thylakoids. In young maize leaf cells, however, such thylakoid dimorphism is not observed in immature chloroplasts of the two cell types, suggesting that the functional division of chloroplasts required for C4 photosynthesis develops as leaf cells mature. Our transmission electron microscopy (TEM) analyses of germinating maize seeds indicate that thylakoids of chloroplasts in young BSCs and MCs are similar in their structures, but Kranz anatomy arises later in the seedling development.

Electron tomography (ET) captures high-resolution images of cells or macromolecular complexes in three-dimension. I propose to elucidate the process of dimorphic chloroplast biogenesis in the MCs and BSCs and assembly of energy-capturing complexes in the thylakoid membrane of developing maize leaf using the advanced electron microscopy. Also, when the suberin layer arises in comparison with Plasmodesmata (PD) remodeling during maize leaf developing process will be examined.

Role of N6-methyladenine DNA Methylation in Abiotic Stress Signaling among Domesticated and Wild Rice Relatives

William Xusheng ZHAO and Wang Kit NG

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

5

Rice (*Oryza sativa* L.) is one of the major staple food around the world. The genus *Oryza* of the Gramineae family includes two cultivated and more than 22 wild species of rice, representing the AA, BB, CC, BBCC, CCDD, EE, FF, GG, KKLL, HHJJ, and HHKK genome types. These worldwide distributed wild rice species constitute an untapped germplasm reservoir for crop improvement. As climate variability increases, extreme temperatures and droughts are likely to impose additional pressure and uncertainty on crop production, which requires effective solutions from all research perspectives. N6-Methyladenine (6mA) DNA methylation has recently been identified as a novel epigenetic marker in eukaryotes, and emerging evidence have suggested a link between 6mA modification and stress responses in plants. The goal of our research is to exploit the genetic diversity of wild rice relatives for their abilities to grow in a wild range of habitats with different abiotic stresses. To achieve this, our lab has obtained 92 accessions of 20 *Oryza* wild species from the National Institute of Genetics in Japan. These reproducing wild rice relatives will be our genetic resources for understanding the roles of 6mA DNA mark in abiotic stress signaling and alleles discovery in wild rice relatives. It is expected that results obtained will facilitate the improvement of cultivated rice for their abiotic stress tolerance in the future.

Molecular Characterization of SH3P2 in Autophagy and Endocytosis

Lanlan FENG, Liwen Jiang and Xiaohong ZHUANG

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

6

Autophagy is a fundamental catabolic process, during which unwanted materials is enclosed in a double membrane structure names as autophagosome and delivered into the vacuole for degradation under stress conditions. Our previous study has identified a novel regulator, SH3P2 (SH3 domain-containing protein2), which contains an N-terminus BAR (Bin/Amphiphysin/Rvs) and C-terminus SH3 (Src Homology 3) domain, plays an essential role in plant autophagosome formation. Upon autophagic induction, SH3P2 is translocated onto the autophagosome membrane and associated with the ATG9 vesicles occasionally. SH3P2 also interacts with ATG8, as well as a plant unique ESCRT (Endosomal sorting complex required for transport) component, FREE1 (FYVE domain protein required for endosomal sorting 1), to regulate the fusion between autophagosome and vacuole. In addition, it has been shown that SH3P2 functions with other endocytic components to involve in the clathrin-mediated endocytosis and cell plate formation. However, the molecular mechanism underlying SH3P2 activity for its switch between autophagy and endocytosis remains largely unknown. Here, we aim to use a combination of cellular, molecular, biochemical and genetic approaches to identify the SH3P2 protein complex and to investigate how they are coordinated during autophagy or endocytosis in plant cells.

Indirect health impacts of 1980–2010 dietary change in China via worsening of particulate matter air quality

Xueying LIU[1], Amos Pui Kuen TAI[1,2], Youfan CHEN[3], Lin ZHANG[3], Gavin SHADDICK[4]

[1]Earth System Science Programme and Graduate Division of Earth and Atmospheric Sciences, Faculty of Science, The Chinese University of Hong Kong, Sha Tin, Hong Kong
[2]Institute of Environment, Energy and Sustainability, and State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Sha Tin, Hong Kong
[3]Laboratory for Climate and Ocean–Atmosphere Studies, Department of Atmospheric and Oceanic Sciences, School of Physics, Peking University, Beijing 100871, China
[4]Department of Mathematics, University of Exeter, Streatham Campus, Exeter, EX4 4QT, UK.

Dietary shifts not only have direct consequences for individual health, but may also lead to "indirect" health impacts through the alteration of environmental quality. To date, metrics linking dietary patterns and environmental sustainability have typically focused on greenhouse gas emissions, but have not included air pollution, which has immediate public health consequences and could provide an important "indirect" link between dietary changes and human health.

To address this research gap, we examine the dietary patterns in China in the early 1980s and 2010s and how their changes and trends could have impacted fine particulate matter ($PM_{2.5}$) air pollution and the associated health cost.

Our results show that dietary changes alone (excluding population rise) from the 1980s to 2010s in China causes a 63% increase (equivalent to 4.0 Tg NH_3 in all of China) in agriculture-related NH_3 emission, while population rise alone causes a 27% increase (1.7 Tg NH_3). Among different forms of dietary changes, the increasing demand for meat contributes to a 2.7 Tg increase in NH_3 emission, while the increasing demand for food crops for direct human consumption causes a 0.9 Tg increase from the 1980s to 2010s. Meat production alone, including raising livestock and growing crops for animal feed, accounts for 67% of the 12.1 Tg agricultural NH_3 emission in 2010.

Increases in annual $PM_{2.5}$ due to dietary shifts alone can be up to about 10 μg m^{-3}, accounting for about 70% of the agriculture-related $PM_{2.5}$ increase and about 20% of the total $PM_{2.5}$ increase over 1980–2010. Of the 1.83 million Chinese premature deaths related to $PM_{2.5}$ pollution in 2010, we estimate that about 5% were related to changes in eating habits from the early 1980s to 2010s. Our work demonstrates a previously unquantified indirect health impact of Chinese dietary change via the worsening of air quality, and serves as an important scientific basis to call for a planetary health framework to incorporate sustainable food production and consumption approaches to safeguard food security and environmental health.

Incense tree *Aquilaria sinensis* - genome, population, and insect interactions

Sean Tsz Sum LAW and Jerome Ho Lam HUI

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

8

The Incense tree *Aquilaria sinensis* (Lour.) Spreng is an agarwood-producing species and is endemic to southern China including Hong Kong. Owing to the overexploitation of its natural populations, the incense tree is currently classified under the "List of Wild Plants Under State Protection (Category II)" in China, and is categorized as "vulnerable" according to the IUCN Red List of Threatened Plants. In my study, a chromosome-level genome and transcriptome assemblies of *A. sinensis* from Hong Kong were assembled. In order to have a better conservation of the Incense tree, low-coverage whole-genome sequencing of >150 individuals from Hong Kong and mainland China were carried out for population analyses. Further, the plant-insect interaction model of this important tree has been established. This is the first high-quality genome assembled as well as plant-insect interaction model for plants in the genus *Aquilaria*, and will be useful for efforts to conserve this economically and ecologically valuable tree species through understanding its biology and evolution.

Role of transient receptor potential ankyrin 1 channels in embryonic stem cell-derived cardiomyocytes

Qianqian DING[1], Rui ZHAO[1], Xianji LIU[1], Suk Ying TSANG[1,2,3]

[1] School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, China.

[2] State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Hong Kong, China.

[3] Key Laboratory for Regenerative Medicine, Ministry of Education, The Chinese University of Hong Kong, Hong Kong, China.

Transient receptor potential (TRP) channels are broadly expressed in a variety of tissues and cell types. They are able to respond to a wide range of stimuli in the cellular environment, which makes them act as cellular vanguard sensors involved in nociception, taste perception, temperature and osmolarity sensation. Among TRP channels, TRP ankyrin 1 (TPRA1) channel was initially described as a cold-sensitive non-selective cation channel expressed in neuron. Recently, emerging evidence indicates that TRPA1 is expressed in various cell types including cardiomyocytes (CMs) and plays an important role in CMs contractile function. Mouse embryonic stem cells (mESCs) are able to self-renew and maintain pluripotency to differentiate into all cell lineages including CMs. In CMs, mitochondria can not only supply energy to cells but also has a key role in the regulation of calcium homeostasis and cell contraction. The function of mitochondrion is tightly related to its morphology, which is determined by continuous fission and fusion, called mitochondrial dynamics. Up till now, there is limited knowledge on how TRPA1 regulates intracellular calcium ($[Ca^{2+}]_i$) and action potential in CMs and whether TRPA1 exerts an effect on mitochondria function. Our preliminary results indicated that mESC-derived CMs (mESC-CMs) expressed TRPA1. In addition, we found that the activities of TRPA1 are positively associated with the Ca^{2+} transients (CaTs) of mESC-CMs. Moreover, TRPA1 activator increased mitochondrial fusion while TRPA1 blocker increased mitochondrial fission. However, the exact mechanism of how TRPA1 regulates the mitochondrial dynamics and function of mESC-CMs is still under investigation. In the future, our research will focus on elucidating how the TRPA1 activity influences mitochondrial dynamics and their effects on the function of mESC-CMs.

Butyrate Protects Endothelial Function through PPARδ

Qinqin TIAN and Wing Tak WONG

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

10

Background: Endothelium plays a key role in maintaining vascular homeostasis and vessel tone by balancing the production of vasodilator and vasoconstrictor. Perturbation of endothelial function will result in impaired vasorelaxation and exaggerated vasoconstriction. Endothelial dysfunction will also lead to atherosclerotic lesion formation and subsequent major adverse cardiovascular events including stroke and myocardial infarction. Butyrate, a short-chain fatty acid (SCFA) produced primarily by bacterial fermentation of fiber in the colon, has been found to possess anti-inflammatory, anti-oxidative and anti-apoptotic properties. Peroxisome proliferator activated receptor (PPAR) is a group of nuclear receptor proteins and a main target of butyrate, including three isoforms: PPARα, PPARγ, and PPARδ. Butyrate has been reported to reduce adipose inflammation in atherosclerosis through activating PPARγ, however, little is known about the effects of butyrate on vascular function and the role of PPARδ in mediating the butyrate-induced vascular function remains unexplored. **Methods and results:** My results showed that butyrate rescued the acetylcholine-induced endothelium-dependent relaxation (EDR) impaired by IL-1β in both the *ex vivo* tissue culture and the *in vivo* high fat diet treatment in *ApoE*$^{-/-}$ mice. Interestingly, knockout of PPAR-δ eliminated the protective effects of butyrate against IL-1β-induced impairment to EDR. I further confirmed that butyrate inhibited IL-1β-induced ROS production in vascular endothelial cells while knockout of PPARδ and inhibition of PPARδ by GSK0660 (PPARδ antagonist) abolished the inhibitory effects of butyrate on ROS production in endothelial cells. Moreover, I found that NOX2, a main enzyme that produces ROS, was inhibited by butyrate treatment at both mRNA and protein levels. While the effects of butyrate on NOX2 expression was abrogated when the endothelial cells were pretreated with GSK0660. Furthermore, I found that IL-1β reduced PPARδ expression which was prevented by butyrate treatment. **Conclusion:** In summary, I demonstrated that butyrate is able to protect against vascular endothelial dysfunction through PPARδ and this study may provide novel mechanistic insights regarding the effective treatment and prevention for vascular disorders.

Functional analysis of SOX9 in choroid plexus development and function

Harry Keng Ioi VONG and Kin Ming KWAN

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

The choroid plexus (CP) is an extensively vascularized neuroepithelial tissue suspended in brain ventricles that forms the core constituent of blood-cerebrospinal fluid (CSF) barrier. Unlike brain vessels forming the blood-brain barrier, CP vasculature lacking complete-belt tight junctions is highly fenestrated such that a functional blood-CSF barrier relies predominantly on securing the permeability of CP epithelium. Yet, how CP epithelium achieves this function remains elusive. We show that specific ablation of *Sox9* in the hindbrain CP results in aberrantly hyperpermeable blood-CSF barrier. The mutant CP shows deficient extracellular matrix anchorage to the basement membrane and fails to define correct apicobasal epithelial polarity. Concomitantly, this perturbs membrane targeting of apical/basal transporters and thus upset the electrolyte balance in the mutant CSF. Mechanistically, SOX9 is required for the transcriptional activation of type IX Collagen in CP. Suppression of collagen α3 (IX) expression during development results in cellular defects that shows remarkable resemblance to *Sox9* mutant phenotype. These data reveal a pivotal cascade of molecular events that construct the blood-CSF barrier and expand our understanding on the modulation of epithelial tissue integrity.

Integrated analysis of lncRNA-perturbated triplets reveals novel prognostic signatures across cancer types

Yunzhen WEI and Dianjing GUO

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

12

Background: Accumulating evidences have shown that long noncoding RNAs (lncRNAs)-mediated ceRNA triplets contribute to cancer development and metastasis. However, identifying significative triplets remains a major challenge for cancer biology. Current studies capture such triplets by co-expression methods but ignore the dynamic changes among factors of the triplets.

Methods: Here, we deeply dissect lncRNA-mediated ceRNA crosstalk from a novel perspective and present a computational framework to identify lncRNA-mediated triplets which were termed as lncRNA-perturbated triplets. We applied the framework to 8 cancer datasets with high tumor purity patient samples in The Cancer Genome Atlas (TCGA) project.

Result: We showed that the paired microRNAs (miRNAs) and mRNAs were widely perturbated by lncRNAs in different cancer types. LncRNA perturbators and lncRNA-perturbated mRNAs showed significantly higher evolution conservation than the other lncRNAs and mRNAs. Importantly, both lncRNA perturbators and lncRNA-perturbated triplets exhibited high cancer specificity. In contrast, we found lncRNA OIP5-AS1 could be recurrently detected in 6 out of 8 cancer types, and it showed higher expression level and affected more mRNAs than that of the cancer-specific lncRNA perturbators. Furthermore, these lncRNA perturbators were found to be significantly enriched in known cancer-related pathways and we also highlighted their potential roles in improving clinical outcomes.

Conclusions: Our study provides a systematical dissection of lncRNA-perturbated triplets and helps understand the underlying molecular mechanisms of lncRNAs in cancers.

Identification of microRNAs and their possible roles in the soybean nodule and root

Kejing FAN and Hon-Ming LAM

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

Soybean miRNAs are important regulators in many plant growth stages. This work was aimed to investigate the roles of miRNAs in symbiotic soybeans roots and mature nodules to identify some common characteristics between the cultivated soybean (C08) and wild soybean (W05).

Cultivated soybean (*Glycine max*) and wild soybean (*Glycine soja*) non-inoculated roots (NOIN), inoculated roots without nodules (RR) and nodules (NOD) were harvested used for RNA and small RNA libraries construction and sequencing. After sequencing, clustering analysis of miRNAs, which was based on their expression pattern, was did to explore the miRNA role in symbiotic soybean roots and mature nodules. From the result, many nutrient starvation responsive miRNAs were induced in symbiotic soybean roots and nodules.

Among the nutrient starvation responsive miRNAs, the expression of miR399 exhibited the highest fold change in RR comparing to NOIN and was selected to do functional analysis. We found miR399 was an important integrator for phosphate and nitrogen in soybean roots and nodules. The induction of miR399 after inoculation is an important strategy for soybeans to maintain the nitrogen fixation and soybean growth.

Short peptides on a long journey: the long-distance signaling in plant

Johnson Wai Ching SIN and Sai Ming NGAI

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

14

Plants utilise a variant of short secreted peptides to modulate growth, development and stress responses. These oligopeptides are typically post-translationally modified, which is crucial to their stability and function. Up to now, over dozens of these peptide families have been categorized, demonstrating the diverse roles of these peptides in cell-to-cell signaling. There is also a growing awareness that plant long-distance signaling mediated by these peptides through xylem sap can coordinate root-to-shoot communication, in the context of plant growth and systemic response against abiotic and biotic stresses. To study these peptides, LC-MS based plant peptidomic analysis is an effective but challenging approach.

In our study, we have performed a comprehensive analysis of soybean xylem sap peptidome through the data-dependent LC-MS approach. From our result, 18 secreted peptide hormones have been identified, including 7 XAP (*XYLEM SAP ASSOCIATED PEPTID*E), 5 CEP (*C-TERMINALLY ENCODED PEPTIDE*), 5 tyrosine sulphated peptides and 1 CLE (*CLV3/ENDOSPERM SURROUNDING REGION RELATED*). Post translation modified variants were identified on these targets, including sulfation on tyrosine, hydroxylation or tri-arabinosylation on proline. Quantitative PCR analysis has revealed that a portion of these peptide precursor genes is expressed specifically in root tissues. Further analysis showed that some of these peptide genes respond to abiotic stress factors.

These results showcased that higher plants employ various post translational peptide hormones in regulating plant development, via facilitating organ-to-organ transmission. Further studies are required to fully decipher such long-distance signaling network in plants.

How SH3P2 interacts with ATG8 in autophagosome biogenesis in Arabidopsis

Shuangli SUN and Kam Bo WONG

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

Autophagy is an intracellular degradation system that delivers cytoplasmic components to the lysosome/vacuoles for degradation and recycling. Numerous studies have done on yeast and mammalian cells, but the autophagosome biogenesis in plants was not clearly understood. We have previously identified a novel protein interact with ATG8, AtSH3P2, involved in autophagosome biogenesis in Arabidopsis. To date, the functional role of this interaction in the biogenesis of autophagosomes is not known. In autophagy, autophagosome formation and cargo recruitment are driven by adaptors and receptors through interactions with lipidated ATG8/LC3 decorating the expanding membrane. ATG8 proteins contain a classical interaction region, which binds receptors and substrates harbouring a AIM/LIR motif. In Arabidopsis, AtNBR1, serve as receptors to target aggregated proteins to autophagosome through binding to ATG8 via AIM motif. However, How does SH3P2 interact with ATG8 at structural basis remains unknown.

As a first step towards structural studies of the SH3P2/ATG8 complex, we determined the NMR structure of ATG8f in apo form. Our results show that ATG8 are highly conserved among species. And by the chemical shift perturbation experiment, we identified the residues in ATG8 that are involved in binding NBR1 peptide and in SH3P2. Three single point mutations near the AIM binding site were created, and these mutations on AtAtg8f blocked its interaction with NBR1 using pull-down assay, suggesting that ATG8f binds NBR1 with its AIM binding pocket. But these mutations did not affect SH3P2-ATG8f interactions as shown by GST pull-down experiments, indicating that SH3P2 may adopt a novel mechanism to interact with ATG8 and the interaction is independent of AIM-binding motif. Besides, we found several surface residues in SH3P2 that weakened the binding to ATG8f by pull down assay and further abolished the interaction using a hextuple mutant by MicroScale Thermophoresis assay. Next, we will use these mutants to test the functional role of SH3P2/ATG8f in Arabidopsis.

NAD tagSeq, a New method for Identification and Characterization of NAD-capped RNAs

Hailei ZHANG[1,#], Huan ZHONG[1,#], Shoudong ZHANG[1,2], Xiaojian SHAO[3], Min NI[1], Zongwei CAI[3], Xuemei CHEN[4,*], Yiji XIA[1,3,5,*]

[1]Department of Biology, Hong Kong Baptist University, Hong Kong, China

[2]School of Life Sciences, Chinese University of Hong Kong, Hong Kong, China

[3]State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, Hong Kong, China

[4]Department of Botany and Plant Sciences, Institute of Integrative Genome Biology, University of California, Riverside, CA 92521, USA

[5]State Key Laboratory of Agricultural Biotechnology, Chinese University of Hong Kong, Hong Kong, China

[#]Authors with equal contribution

A eukaryotic mRNA has been known to have a m^7G cap that protects mRNA from degradation and mediates almost all other aspects of gene expression. Some RNAs in *E. coli*, yeast, and mammals were recently found to contain an NAD^+ cap. We have developed a new method, NAD tagSeq, for transcriptome-wide identification, quantification, and characterization of NAD^+-capped RNAs. The method uses an enzymatic reaction and then a click chemistry reaction to label NAD-RNAs with a synthetic RNA tag. The tagged RNA molecules can be identified by single-molecule direct RNA sequenced using the Oxford nanopore sequencing technology. NAD tagSeq allows more accurate identification and quantification of NAD-RNAs as well as reveals the whole sequences of NAD-RNAs. Using NAD tagSeq, we found that NAD-RNAs in *Arabidopsis* were produced by at least several thousand genes, most of which are protein-coding genes. The top 2,000 NAD-RNA-producing genes are enriched in the biological processes of photosynthesis, protein synthesis, and stress responses. The NAD-RNAs in *Arabidopsis* generally have the same overall sequence structures as the canonical m^7G-capped mRNAs, although a majority of them appear to have a shorter 5' UTR. The wide-spread presence of NAD-RNAs indicates that they play an important role in gene regulation.

The role of *AtMYB30* in abiotic stress response networks in Arabidopsis

Yulong GONG and Jianhua ZHANG

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

As sessile organisms, plants are continually saddled with miscellaneous abiotic stress conditions by the inorganic milieu. To overcome such predicament, plants have evolved various adaptation mechanisms, of which the molecular basis can be divided into downstream effectors and upstream regulators, such as kinases and transcription factors (TFs). To find novel regulators in stress response network, 79 candidate genes were chosen from 2 Arabidopsis transcriptome databases under dehydration stress. Fortunately, some novel phenotypes of promising lines were found, especially for the mutant line of *AtMYB30* which shows hypersensitivity to salt stress, cadmium stress and more importantly, to oxidative stress.

AtMYB30 belongs to the subfamily 1 *R2R3-MYB*s, it was first reported as a key regulator in biotic stress response network, yet evidences rises that it may also participate in some abiotic stress response pathways. DAB staining and GSH assay have indicated the sensitivities to hypoxia and cadmium stress attribute largely to the increased ROS level. *AtMYB30* was reported to regulate the oxidative stress response pathway by control calcium flow, while our studies have shown that it also functions in phytohormone (like ABA and NO) dependent manners. Furthermore, several upstream regulators of *AtMYB30* is disclosed by Y1H assay. Further analyses indicate that *AtMYB30* may be trans-regulated by some subfamily 4 *R2R3-MYB*s, and this hypothesis has been proved by qPCR analysis and Luciferase assay This study may give us new insights on the roles of *AtMYB30* in plant abiotic stress regulation network, and it may give us new clues to solve abiotic stresses faced by agriculturally, economically and environmentally important plants.

Taking together, we are among the first teams who have disclosed that *AtMYB30* is a key regulator in oxidative stress, of which the function is also ABA and NO responsive. And the upstream regulators unveiled in this study can help to study the role of AtMYB30 as a key knot of inter-stress response pathways.

An efficient ChIP-seq method for genome-wide identification of protein-DNA interactions in rice protoplasts

Wei HAO and Silin ZHONG

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

18

DNA-binding proteins and histone modifications are essential for gene transcriptional regulation. Chromatin immunoprecipitation followed by sequencing (ChIP–seq) is the most powerful tool to profile the protein-DNA interactions genome-wide. Using transgenic plants expressing target protein with an epitope tag for assaying protein-DNA interactions is time and labor-consuming.

Here, we applied an efficient ChIP-seq method using rice protoplast system. We optimized the ChIP-seq protocol, including the transfected DNA amount and the preparation of sequencing library. We show that this method significantly reduces the time, about 2 days for histone and 3 days for transcription factor ChIP-seq. We also demonstrate that the ChIP-seq results of a transcription factor are reproducible and reliable. We reveal that our histone ChIP-seq data are consistent with previous studies. Finally, we show that the results using our method is comparable to that obtained from the transgenic plants of *Arabidopsis*.

Our ChIP-seq method using rice protoplast system facilitates the study of genome-wide mapping of DNA-binding proteins and epigenetic marks in *vivo*.

Structural variation and gene presence-absence variation detection in plants

Andy Yuxuan YUAN and Ting Fung CHAN

State Key Laboratory of Agrobiotechnology (CUHK)

School of Life Sciences, The Chinese University of Hong Kong

Structural variations (SVs) are forms of genetic variation, which play important roles in evolution and functional diversity. SVs can affect phenotypes by either changing the structure or content of particular genes. During the past decades, the study of SVs in plants has been limited owing to the imprecise technologies and methods. With the development of technologies and methods, the characterization of SVs in plants has been viable.

SVs can change gene dosage in one individual with some gene duplicated and some deleted. Recently, there is an increasing awareness that the genetic information encoded in one individual cannot represent the whole genetic features in the species owing to gene presence and absence variations, copy number variations and other insertions/deletions. To characterise the genetic features in a species and explore gene variations between individuals, pangenome studies can be conducted. A pangenome describes the whole gene set in a species, involving genes present in all individuals (core genes) and genes present only in some individuals (variable genes). Mining variable genes and associating them with useful agronomic traits could help plant breeders to produce advanced crop varieties to feed the world.

In my research, I mainly focus on SV studies using the latest sequencing technologies and optical mapping with some methods being developed.

Acyl-CoA-binding proteins play important roles in plant lipid metabolism

Zehua GUO and Mee-Len CHYE

State Key Laboratory of Agrobiotechnology (CUHK)

School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China.

20

Oryza sativa (rice) seeds represent staple food for many people in Asia. Past reports have indicated that *Arabidopsis thaliana* acyl-CoA-binding proteins (ACBPs) are important in seed development. We now show that rice OsACBP2 is also important in seed development. *OsACBP2* mRNA accumulated in embryos and endosperm of germinating seeds, while β-glucuronidase (GUS) assays showed GUS expression in *OsACBP2pro::GUS* rice embryos, and the scutellum and aleurone layer of germinating seeds. Using *osacbp2* mutants and transgenic rice overexpressing OsACBP2 (OsACBP2-OE) in our investigations, we observed that *osacbp2* was retarded in germination, while OsACBP2-OEs exceeded the controls in germination, seedling growth, grain size, and grain weight. Transmission electron microscopy of OsACBP2-OE mature seeds detected oil body accumulation in the scutellum cells, while confocal laser scanning microscopy revealed oil accumulation in the aleurone tissues. As OsACBP2-OE seeds contained higher triacylglycerol and long-chain fatty acid content over the control, OsACBP2 appears to be a promising candidate for enriching seed nutritional value.

Evolutionary Timeline, Genomic Innovation and Pre-Adaptation Underlying the Lifestyle Diversity in *Rhizobiales*

Sishuo WANG and Haiwei LUO

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

21

Members of the *Rhizobiales* include those capable of nitrogen fixation in nodules as well as pathogens of animals and plants, and this lifestyle diversity has important implications for agricultural and medical research. Leveraging large-scale genomic data, we infer that *Rhizobiales* originated as a free-living ancestor ~1,500 million years ago (Mya), and later, the emergence of host-associated lifestyles generally coincided with the rise of their terrestrial plant and animal hosts. Particularly, the first nodulating *Rhizobiales* lineage arises from either *Bradyrhizobium* or *Azorhizobium*, coinciding with the emergence of nodulating plants ~145-110 Mya. The rates of lifestyle transitions are highly variable; nodule-association is more likely to be lost than gained, whereas animal-association likely represents an evolutionarily dead end. Among the genes that facilitate successful transitions to major nodulation lineages (*Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*/*Ensifer*, and *Rhizobium*), the *nod* and *nif* clusters for nodulation and nitrogen fixation, respectively, were repeatedly acquired during each transition, while the *fix*, *dct* and *phb* clusters involved in energy conservation under micro-oxic conditions were already present before each transition. Type III, IV, and VI secretion systems, which promote nodule formation and rhizobia invasion, were either pre-adapted or acquired during transition in a lineage-specific pattern. Pre-adapted traits may have also contributed to the emergence of the animal pathogens (*Bartonella* and *Brucella*) in *Rhizobiales*, among which iron transporter and class Ib ribonucleotide reductase may help overcome iron limitation in mammalian bloodstream. Our study suggests that increased eukaryote diversity likely drives lifestyle diversification of bacteria, and highlights pre-adapted traits facilitating the origin of host-association.