香港中文大學 CUHK
農業生物技術國家重點實驗室

SKLA / IPMBAB SEMINAR SERIES | 2020

IPMBAB

7 NOV 2020 (SAT) | 8:45am - 1:15pm | HONG KONG

Abstract Booklet

Elucidating protein structures of rice ACBPs

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Speaker

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Our laboratory (http://www.biosch.hku.hk/staff/mlc/mlc.html) has been working on plant acyl-CoA-binding proteins (ACBPs) the past decade using genetic, biochemical and plant biology approaches. ACBPs are a family of proteins that facilitate the binding of long-chain acyl-CoA esters at a conserved acyl-CoA-binding (ACB) domain. ACBPs transport acyl-CoA esters, form intracellular acyl-CoA pools, and regulate lipid metabolism. In *Arabidopsis thaliana,* a family of six ACBPs has been demonstrated to function in stress and development. In *Oryza sativa* (rice), we have previously reported the apo structures of OsACBP1 and OsACBP2 (Guo *et al.,* 2017). Herein, we expand our knowledge on OsACBP2 interaction with acyl-CoA esters. We report on the structure of OsACBP2 in complex with C18:3-CoA ester, which represents the first ligand-bound plant ACBP complex structure (Jing *et al.,* 2020). This complex diffracted at 3.5 Å and revealed six molecules of OsACBP2 in an asymmetric unit. The residues Y33, K34, and K56 of OsACBP2 play a crucial role in binding the CoA group. The phenyl group in Y33 stabilizes the adenine group in coenzyme-A *via* forming a π-π interaction. Interestingly, OsACBP2 was also demonstrated to bind the fatty acyl moiety of C18:3-CoA with a cooperating molecule. The residues N23, L27, K52, and Y55 in one molecule of OsACBP2 cooperate with L26, L27, A59, and A62 from another anchoring the fatty acyl group. Furthermore, size-exclusion chromatography-coupled multi-angle light scattering assays indicated OsACBP2-bound C18:3-CoA as a monomer, and supports a novel interaction model between a fatty acyl-CoA ester and the ACB domain(s).

Brassinosteroid regulates nitrate remobilization under nitrogen starvation condition in *Arabidopsis*

Yuling ZHOU and Junxian HE

State Key Laboratory of Agrobiotechnology (CUHK) and

School of Life Sciences, The Chinese University of Hong Kong

Speaker

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Nitrogen (N) is one of the most important nutrients for plants, controlling almost all aspects of plant growth and development, and nitrate is the main source of nitrogen in the soil. Phytohormones modulate nitrate regulatory networks and metabolism. However, how the plant steroid hormone brassinosteroid (BR) regulates plant nitrogen response and signaling is poorly understood. Recent studies suggest that the root foraging response under N deficiency relies on a systemic regulation of BR biosynthesis or BR signals, suggesting a considerable potential of manipulating BR biosynthesis or signaling in genetical engineering of plants with improved N use efficiency. However, how BR regulates N use efficiency remains unclear.

Here, we found that under nitrogen starvation condition, the younger leaves of bin2-1, a gain-of-function mutant of the GSK3-like kinase BIN2, are greener than that of wild type. We hypothesize that BIN2 may play a role in regulating N use efficiency, particularly remobilization of nitrogen from old to young leaves.

In support of our hypothesis, qRT-PCR analysis showed that the transcript levels of NRT1.7, NRT1.11 and NRT1.12, involved in remobilization of nitrate, are upregulated in bin2-1 under nitrate starvation condition. By yeast two-hybrid assay we found that BIN2 can interact with NLP7, a key transcription factor (TF) of nitrate signaling pathway. As an important kinase, BIN2 has been shown to participate in a wide range of developmental processes through phosphorylating a multitude of substrates, including many TFs. Therefore, we hypothesize that BIN2 may mediate BR-regulated N remobilization by interacting and phosphorylating NLP7. To test this hypothesis, we have carried out an in vitro kinase assay and found that BIN2 can indeed phosphorylate NLP7. Consistently, we have also detected five BIN2 phosphorylation sites in NLP7 by Mass Spectrometry. In the future, we will perform more functional studies to illustrate how BIN2 interacts with NLP7 to modulate nitrate remobilization under nitrogen starvation.

The Histone Modification H3K4me3 Marks Functional Genes in Soybean Nodules

Qianwen WANG and Hon-Ming LAM

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Speaker

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Soybean could develop the specialized organs called root nodules for symbiotic nitrogen fixation. Studies showed that developmental processes are regulated by epigenetic marks such as histone H3 lysine 4 trimethylation (H3K4me3). Here we characterized the high-confidence transcriptomic data and genome-wide patterns of H3K4me3 marks in soybean roots and mature nodules symbiotic with *Sinorhizobium fredii*. As expected, changes in H3K4me3 levels were positively associated with the transcription levels of functional genes in the nodules. The up-regulation of H3K4me3 levels was not only present in leghaemoglobin and nodulin-related genes, but also in most of the genes involved in nitrogen and carbon metabolic pathways. On the contrary, a loss of H3K4me3 marks was found in several key transcription factor genes, including four *GmWRKYs*, and was correlated with the down-regulation of the defence-related network in nodules. Hence we have uncovered a regulatory network that could contribute to nodule maintenance. In addition, genes regulating the transmembrane transport of metal ions, phosphates, sulphates, peptides, and sugars were differentially modified with H3K4me3, revealing the complex regulation of transmembrane transportation during symbiosis. All these findings demonstrate massive reprogramming of gene expressions via alterations in H3K4me3 levels in the genes in mature soybean nodules, thus supporting the role of H3K4me3 in maintaining nodule functions.

Exploiting wild rice germplasms in abiotic stress signaling for rice improvement

Xusheng ZHAO and Wang Kit NG

State Key Laboratory of Agrobiotechnology (CUHK) and

School of Life Sciences, The Chinese University of Hong Kong

Speaker

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As one of the major staple food crops worldwide, rice (Oryza sativa L.) has more than 20 wild relatives within the same genus, which form huge genetic resources for crop improvement. With continuous population growth and intensifying climate extremes, abiotic stresses (such as drought, heat, salinity and nutrient deficiency) are expected to impair crop productivity, thereby threating food security. To cope with these environmental limitations and guarantee food security, there is an urgent need for breeding “climate-ready” crop cultivars with improved tolerance to abiotic stresses. So far, our lab has reproduced 41 accessions of 13 wild species which origin from the whole tropical and subtropical regions. Through phenotypes screening for abiotic stress responses, some specific germplasms are selected for more in-depth physiological and transcriptome (mRNA-seq) characterizations. In addition, emerging evidence have shown that N6-methyladenine (6mA) DNA methylation levels were significantly changed in response to abiotic stresses, while C5-cytosine DNA modification levels maintained stable. Therefore, identifying the change in 6mA DNA level among various wild rice relatives (WRR) and their relationship with abiotic stress tolerance will facilitate further molecular characterization of the WRR in response to abiotic stresses. Such information will help to identify potential epialleles and alleles for use in cultivated rice improvement.

Effects of dryland irrigation on regional climate and air quality in Northwest China: A modeling study

Oscar Hiu Fai TAM and Amos Pui Kuen TAI

Earth System Science Programme and Graduate Division of Earth and Atmospheric Sciences and
State Key Laboratory of Agrobiotechnology (CUHK)

Speaker

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Investigating the modification of regional climate and air quality by irrigation is particularly important for semiarid regions such as Northwest China, where water resources are limited. Such modification can be substantial when compared with the relatively dry climatic background. Therefore, optimization of water use by striking a balance between irrigation, water availability, crop yields, regional climate and air quality provides useful insights to the future development of irrigation practices, as well as agricultural approaches at large. In this study, we took a modeling approach to address such issues by using a state-of-the-art climate-chemistry-land model called WRF-GC, which couples the widely used Weather Research and Forecast Model (WRF) with the GEOS-Chem chemical transport model, with various options to represent land surface and agricultural processes. With its ability to simulate how weather and air pollutants evolve with time, we studied the effects of implementing different irrigation schemes including flooded, sprinkler, and drip irrigation on regional climate and air quality. We found that flooded and sprinkler irrigation practices greatly reduce sensible heat flux but increase latent heat flux. The induced cooling by flooded irrigation cools the surface up to 6°C as inferred from the averaged drop in near surface air temperature over daytime. Consequently, planetary boundary layer height decreases, which inhibits vertical mixing of air pollutants and deteriorates surface air quality. In particular, surface NO_x concentration increases by ~0.7 ppb (26%) if flooded irrigation is adopted over southeastern Gansu. On the contrary, air quality worsening is minimized if drip irrigation is adopted, which is attributable to the minimal changes in regional climate. We concluded that drip irrigation is likely the most preferable option for semiarid agriculture, not only because of its water-saving potential but also its minimal impacts on the atmospheric environment.

How do leaf traits regulate leaf-to-air temperature difference?

Zhengfei GUO and Jin WU

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Speaker

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Leaf temperature and associated leaf-to-air temperature difference (ΔT) govern the plant metabolic rate, and thus are important indicators of plant stress and health. Currently, it is widely observed that ΔT varies largely within a day and across plant species and diverse biomes, and is regulated by both abiotic and biotic factors. Although some mechanistic models (e.g. leaf energy balance) have been used to quantify abiotic regulations on ΔT, critical challenges remain with accurate mechanistic representations or comprehensive evaluations of biotic controls on ΔT dynamics. To quantify biotic roles in regulating ΔT variability, we combined a coupled biochemical photosynthesis-stomatal conductance model with a leaf energy balance model, in which there are six leaf traits, i.e. leaf emissivity, visible and near-infrared light absorptance, leaf size, leaf maximum carboxylation rate scaled to 25 °C ($V_{c,max25}$), and Medlyn type's stomatal slope (g_1). With this integration, we first performed sensitivity analysis to quantify the extent to which leaf traits mediate ΔT variability. We then quantified the relative importance of each trait for ΔT, and explored whether their relative importance varies at the diel timescale. We last evaluated our modelling results with *in-situ* observations through both literature data and field campaigns conducted at three forest sites spanning from a high-latitude temperate forest to two low-latitude tropical (moist and dry) forests. Our results show that: (1) leaf traits largely mediate ΔT variability, with noon-time ΔT of a summer clear-sky day varying from -3 °C to 12 °C; (2) leaf size, $V_{c,max25}$, and g_1 are the three most important traits, and their relative importance in ΔT regulation is not static but varies strongly across the diel course; (3) simulated trait-ΔT relationships strongly agree with field observations. Our study improves the quantification and process understanding of biotic controls of ΔT variability, while providing a trait-based representation of leaf energy balance that can be incorporated into terrestrial biosphere models for improved simulations of plant thermoregulation across species and vegetation response to climate change.

Dynamic epigenome changes in response to light in *Brachypodium distachyon*

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Speaker

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Light plays an important role in many plant biological processes such as photosynthesis and photomorphogenesis. In this study, we applied RNA-seq, ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) as well as histone modification and transcription factor ChIP-seq (Chromatin Immunoprecipitation Sequencing) to study how transcriptional regulation could be affected by epigenome in *B. distachyon* under controlled light and extended darkness conditions. We have identified 8,400 differentially expressed genes (DEGs), and they are enriched in photosynthesis Gene Ontology (GO) terms. Over 20% of the open chromatin regions of the total genes decreased after extended darkness treatment, indicating that light has a dramatic impact on chromatin accessibility. We also found that differential H3K4me3 andH3K9ac modifications enriched in gene loci associated with photosynthesis and other light-dependent reactions. We further identified a candidate transcription factor BdHY5-LIKE that targets these light reaction genes. Together, our results indicated the light-induced changes of chromatin accessibility and two histone modifications contributes to the transcriptional regulation of BdHY5-LIKE in photosynthesis.

The Pollen Tube Tip-Vesicles

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Speaker

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The rapid polar growth of pollen tube is maintained by highly dynamic transport vesicles participating in endocytosis and exocytosis in the tip region (Grebnev *et al*., 2017). However, the nature, identity and function of these tip-vesicles (TVs) remain elusive. Here we aim to study the nature and function of TVs. To obtain high-resolution maps of TVs, we performed 3D electron tomography (ET) analysis (Cui *et al*., 2019) on lily pollen tube tip region. Preliminary results show that TVs contains different populations according to their size and appearance, including electron-translucent large vesicles (LVs, 190-280 nm), dense vesicles (DVs, 70-180 nm), mini vesicles (MVs, 30-40 nm) and clathrin-coated vesicles (CCVs, 50-100 nm). Various fusion profiles between vesicles and the plasma membrane (PM) implying exocytosis or endocytosis were also observed and further analyzed by 3D ET. Interestingly, extensive apoplastic tubular structures, probably contributing to the formation of extracellular vesicles (EVs) (Cui et al., 2019), could be observed along the apical dome of the PM. The 3D ET analysis on Arabidopsis pollen tube tip region also reveals similar heterogeneity of TVs. To obtain close-to-native ultrastructure of TVs, we recently used Cryo-FIB (Focused Ion Beam) milling (Schaffer *et al*., 2019) to prepare lamella from pollen tube for cryo-ET analysis. The cryogenic ultrastructure would reveal the architecture of TVs at an unprecedented level. Future studies include TVs isolation and generation/screening of monoclonal antibodies against specific membrane components of TVs, aiming at developing molecular tools to dissect the nature and function of distinct TVs in germinating pollen tubes.

Friendly is required for membrane depolarization-induced mitophagy in Arabidopsis

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Speaker

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The oxidative environment within the mitochondria makes them particularly vulnerable to proteotoxic stress. To maintain a healthy mitochondrial network, eukaryotes have evolved multi-tiered quality control pathways. If the stress cannot be alleviated, defective mitochondria are selectively removed by autophagy via a process termed mitophagy. Despite significant advances in metazoans and yeast, in plants, the molecular underpinnings of mitophagy are largely unknown. Here, using time-lapse imaging, electron tomography and biochemical assays, we show that uncoupler treatments cause loss of mitochondrial membrane potential and induce autophagy in Arabidopsis. The damaged mitochondria are selectively engulfed by autophagosomes that are ATG5 dependent and labelled by ATG8 proteins. Friendly, a member of the Clustered Mitochondria protein family, is recruited to the damaged mitochondria to mediate mitophagy. In addition to the stress, mitophagy is also induced during de-etiolation, a major cellular transformation during photomorphogenesis that involves chloroplast biogenesis. De-etiolation triggered mitophagy is involved in cotyledon greening, pointing towards an inter-organellar cross-talk mechanism. Altogether our results demonstrate how plants employ mitophagy to recycle damaged mitochondria during a stress condition and development.

A patch-forming protein involved in the degradation of chloroplast and mitochondria OEM tail-anchored proteins

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Speaker

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In our study, we found an uncharacterized cytosol protein, At5g42220, interacted with both TOC and TOM traslocons which was the import machinery of chloroplast and mitochondria proteins respectively. We named this protein PFP (patch-forming protein) as the diffuse At5g42220 could transform into puncta in the cell under stress conditions. There was an ubiquitin-like (UBL) domain in its N-terminus of PFP, which was involved in ubiquitin-proteasome system for protein degradation in various cellular processes.

Knock-out of PFP did not make any changes to plant growth, but PFP overexpression lead to plant death. TEM study of chloroplast ultrastructure in PFP inducible expression transgenic plants showed the chloroplasts biogenesis was impaired when PFP was expressed, which lead to plant chlorosis and death. Additionally, pfp mutants de-etiolated inefficiently, displaying reduced survival rates linked to delayed organellar differentiation. Our further study will also investigate the effects of PFP on mitochondria biogenesis. Thus, we hypothesized that PFP played an important role in chloroplasts biogenesis and probably regulated TOC and TOM constitutive degradation to maintain chloroplasts and mitochondria proteostasis. To prove this, we found accumulations of Toc33 and Tom20-2 in pfp mutants compared to WT. Treatment of MG132, a proteasome inhibitor, increased Toc33 accumulation in WT plants, but did not make significant change in pfp mutants, which indicated that PFP promoted Toc33 degradation through the proteasome.

Previous studies found a chloroplast-associated degradation pathway, in which TOC translocons were ubiquitinated by a chloroplast outer membrane E3 ligase SP1, and retro-translocation of TOC translocons out of the membrane were then manipulated by chloroplast outer membrane SP2 to act as a channel and cytosolic CDC48 to provide driving force. In our study, we found PFP was able to interact with both SP2 and CDC48. Except that, PFP also co-immigrated with CDC48 and Toc33 or Tom20-3 in BN-PAGE analysis. Our preliminary study found CDC48 also interacted with TOM translocons at mitochondria outer membrane in Arabidopsis. Thus, we postulated that PFP was involved in a common regulation pathway for TOM and TOC translocons degradation through the proteasome.

Molecular Characterization of ATG9 Vesicles in Plant Autophagy

Yingfei QUAN, Liwen JIANG and Xiaohong ZHUANG

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Speaker

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Macroautophagy, also known as autophagy, is a fundamental degradative pathway essential for cellular homeostasis. During autophagy, a set of autophagy-related proteins are recruited to the pre-autophagosomal structure (PAS) for *de novo* formation of a double-membrane vesicle called an autophagosome. Vesicles containing ATG9 proteins has long been regarded as one important membrane source for autophagosome biogenesis. In yeast and animal cells, loss of functional ATG9 impairs autophagosome formation with few autophagosomes labeled by the autophagosome marker ATG8. Differently, remarkable ATG8e-positive tubular structures are accumulated in *atg9* mutant plants, which are directly connected to the Endoplasmic Reticulum (ER). However, the compositions of ATG9 vesicles and how they contribute to autophagosome formation is still unclear in plants. To gain more insights into the regulatory mechanism of plant ATG9 vesicles, I firstly performed organelle fractionation to purify the ATG9 vesicles using an Arabidopsis cell line expressing FLAG-HA-ATG9. Our data showed that ATG9 vesicles are largely separated from other known compartments, suggesting they have a unique subcellular distribution in plant cells. Furthermore, via a pull down assay followed by Mass spectrometry (MS) analysis, I have successfully identified several ATG9-associated candidates. I characterized one protein called Trs85, which belongs to the TRAPP (transport protein particle) complex to function in endomembrane trafficking. By subcellular analysis, I found that Trs85 as well as other TRAPP subunits are closely associated with ATG9 and ATG8. Taken together, our data points to an intimate connection between ATG9 vesicles and endocytic traffic during plant autophagy.

Small peptide analysis of soybean using data-independent acquisition mass spectrometry

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Speaker

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One common approach of LC-MS/MS is the conventional data-dependent acquisition (DDA) tandem mass spectrometry, which is used in protein identification and quantification of complex samples. However, precursor ions of higher intensity level are more likely to be selected for fragmentation using DDA, which may result in redundant precursor selection and hinder the detection of low-intensity precursor ions. For the identification of small peptides, especially those that are encoded within what previously mistook as long 'non-coding' RNAs, establishing a complete protein profile is of necessity. Therefore, another approach – data-independent acquisition (DIA) overcomes the deficiencies of DDA and provides an alternative option in precursor selection, which is unbiased. In DIA, all the precursor ions within an isolation window (m/z) are proceeded to fragmentation, which gives a detailed but complex spectrum.

As DIA allows multiple precursor ions selection, the resulting fragment ions spectrum is much more complex than the DDA counterpart, thereby making it far more challenging in data analysis. Many DIA data analysis tools have been developed, mainly divided as two groups – library-based and library-free. The number of identified peptides found in DIA can be nearly 20% more than that found in DDA. To evaluate the performance of these tools, we obtained DIA-MS data from soybean root samples of two germplasms: the cultivar Williams 82 and wild W05. Lists of identified peptides generated using different software platforms were matched to an in-house soybean lncRNA database, which contains a list of confirmed lncRNAs in soybean root samples. As different approaches and peptide search engine are used, the results from the software showed some variations, especially the lists of identified peptides. Therefore, combining the results from several software is necessary but there is no well-developed and integrated platform. In future work, we will collect DIA mass spectrometry data from more germplasms to help us develop an integrated pipeline for DIA.

The Landscape of Accessible Chromatin in *Artemisia annua* Glandular Trichome and Leaf

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Speaker

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Glandular trichome (GT) is the dominant site for artemisinin production in *Artemisia annua*. Several critical genes involved in artemisinin biosynthesis are specifically expressed in GT. However, the molecular mechanism of differential gene expression in GT vs. other tissue types remains elusive. Chromatin accessibility, defined as the degree to which nuclear molecules are able to interact with chromatin DNA, reflects gene expression capacity to certain extent. Here, we used ATAC-seq to investigate the landscape of chromatin accessibility in *Artemisia annua* leaf and GT. We revealed that long-range regulation may play an important role in regulating GT gene expression. In addition, GT harbors more accessible regions (ACRs) that are likely associated with GT-related biological functions, such as stress response and secondary metabolism. We found GT-specific artemisinin biosynthetic genes like *DBR2* and *HMGS* had more accessible ACR in GT compared to that in leaf. In addition, we predicted and experimentally validated two MYB transcription factors involved in artemisinin biosynthetic gene regulation. Therefore, we dissected the chromatin accessibility landscape in *Artemisia annua*, providing insight into epigenetic regulatory mechanism of gene expression.

Homeobox genes and microRNAs evolution in invertebrates

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Speaker

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Homeobox genes are crucial transcription factors in regulating different developmental processes and can serve as genetic markers of large-scale genomic changes in evolution. The largest group of homeobox genes in animals is the ANTP class. Previous comparative genomics studies deduced that the Hox, ParaHox and NK genes were clustered in metazoan ancestor, and has then been dispersed into at least four chromosomal clusters by the time of the bilaterian ancestor. The arrangement of these clusters in different invertebrates remains to be fully elucidated.

On the other hand, microRNAs are 21-24 nucleotides of non-coding RNAs that are potent regulators of gene expression at post-transcriptional level and genome stability, and yet, their targets in animal evolution are poorly understood. To date, most of our understanding of microRNA regulation and their potential contribution to evolution mainly draws from a few model organisms, including the human *Homo sapiens*, mouse *Mus musculus* and fruit fly *Drosophila melanogaster*. All these animal models are triploblastic bilaterians, and the diploblastic non-bilaterians, such as the cnidarians with the absence of mesoderm or muscle are understudied in this regard.

In this study, the homeobox genes in jellyfish genomes, mollusc genomes and arthropod genomes were annotated and the results shown that the Hox, NK, and Hox-like genes were linked in the jellyfish genomes. While in the millipede, horseshoe crab and oyster genomes, they are separated. These results indicated that the ANTP gene cluster evolution took very divergent evolutionary pathways in cnidarians and bilaterians. miR-100, previously thought to be the miRNA conserved between cnidarians and bilaterians seems to have been lost in the scyphozoan jellyfish. Only miR-2022 and miR-2030 appear to be conserved in cnidarians. These findings provide insights into the understanding of evolutionary pathways of both bilaterians and cnidarians.

Functional Link between Cerebellum and Pathogenesis of Autism

Sum Yi MA and Kin Ming KWAN

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Speaker

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Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disorders that affect up to 1 in 100 world individuals. People with autism display an array of symptoms encompassing impairment in social communication, repetitive behaviour, perception and memory and motor defects. No theory has suggested a single underlying neuropathology to explain these spectra of symptoms. The cerebellum, conventionally associated with motor function in human, has increasing evidence showing that it may contribute to ASD by affecting the social circuitry. However, there is limited study focusing on the cerebellar in autism so far. In our study, we aim to investigate the cerebellar structure and determine the expression of rate-limiting GABAergic decarboxylases and transporters in GABA metabolism of the autism cerebellum. C57BL/6 mice were intraperitoneally injected with a dosage of 500 mg/kg valproic acid (VPA) on an embryonic day 10.5 for autistic behavioural induction. The autistic mouse models displayed sociability deficit and repetitive behaviour. We found that exposure of VPA led to neural tube deformation, decreased cerebellar size and white matter area. For teenage mouse models with autistic behaviour, they showed reduced expression of glutamate decarboxylases and GABA transporter in the cerebellum by both immunoprotein and immunofluorescence assays. Thus our findings suggest that cerebellum impairment could be an etiology of environmentally induced autism. Changes in cerebellar structure and the altered GABA metabolism in the cerebellum provide targets for future clinical studies in idiopathic autism patients.

Lifestyle evolution in *Bradyrhizobium*

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Speaker

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Bradyrhizobium is a lineage of nitrogen-fixing symbiotic bacteria that is important to the ecological success of thousands of legume species and served as a model microorganism for studying host–microbe symbiotic interactions. Nevertheless, in addition to nodulating and symbiotic nitrogen fixation, the slow-growing bacteria still has other lifestyles. For example, a recent study found that non-symbiotic *Bradyrhizobium* ecotypes dominate North American forest soils. Besides, a free-living N_2-fixing *Bradyrhizobium* was isolated from roots of sweet potato.

To date, however, only few non-symbiotic strains were isolated from soils. Most of strains available in public databases are symbiotic that derived from legume nodules. Yet, global soil bacteria survey found that *Bradyrhizobium* is one of the most abundant and widespread taxa in soils across the world. These findings demonstrate how focusing research on economically important microorganisms can bias our understanding of bacteria function and suggest that the influence of this genus likely extends well beyond facilitating agriculture.

Here we designed an efficient selective medium and isolated 93 *Bradyrhizobium* strains from different soil samples. Of these 93 strains, 11 have both nodulation (*nod*) and nitrogen-fixation (*nif*) genes, three only have *nif* genes, and the rest 79 strains contain neither *nod* nor *nif* genes. By constructing phylogenetic tree with 221 *Bradyrhizobium* genomes deposited in the NCBI database, we revealed that *Bradyrhizobium* likely originate from free-living ancestors. The free-living to nodulating transition happened 8 times independently within this genus, but the symbiotic lifestyle was not stable, and the symbiotic strain was easily shifting back to free-living lifestyle. With gene grouping and annotation combined with phylogenetic tree, gene gains or losses during lifestyle transitions could be revealed. Phylogenetic analysis of *nif* genes showed that *nif* genes clearly separated based on lifestyles. Moreover, the gene arrangement on *nif* islands was highly conservative among members in the free-living group, suggesting horizontal gene transfer facilitating rapid expansion of the free-living N_2-fixing lifestyle.

TRPC3 contributes to triple negative breast cancer progression

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Speaker

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Triple-negative breast cancer (TNBC) does not respond to hormonal therapy medicines or medicines that target HER2. New targets for effective molecular-based therapy are urgently needed. Previous study has demonstrated that canonical transient receptor potential isoform 3 (TRPC3), a calcium-permeable non-selective cation channel, is upregulated in breast cancer biopsy tissues when compared to normal breast tissues. Since intracellular Ca2+ homeostasis is closely relevant to tumorigenesis and tumor progression, the role TRPC3 plays in breast cancer progression is waiting to be understood.

Previously, utilizing MDA-MB-231 cells, a type of TNBC cancer cells, we have demonstrated that TRPC3 contributes to the cell proliferation of TNBCs. We applied Pyr3 and dominant negative of TRPC3 to block TRPC3. Proliferation assays showed that blocking TRPC3 could attenuate proliferation in MDA-MB-231. Ras GTPase-activating protein 4 (RASA4) is a Ca2+-promoted Ras-MAPK pathway suppressor. We observed that amount of RASA4 located on the plasma membrane decreased following TRPC3 blockade. Activation of MAPK pathways was also confirmed with western blot. Thus, we suggested that TRPC3 regulates the Ca2+ homeostasis and down-stream located Ras-MAPK pathway to promote proliferation in TNBC MDA-MB-231 cells.

In our current study, to further elucidate the role of TRPC3 in breast cancer progression, stable MDA-MB-231 cell line with TRPC3 knockdown (MDA-MB-231-shTRPC3) was established by lentiviral vector-mediated transduction. Long-term knockdown of TRPC3 inhibited MDA-MB-231 cell migration as measured by wound healing assay. Importantly, TRPC3 was found to regulate the translocation of the calcium-sensitive transcription factor NFATc1 in MDA-MB-231. Blocking TRPC3 and knocking down of TRPC3 both caused the translocation of NFATc1 from the nucleus to the cytosol. Previous studies suggested a link between NFATc1 signalling and cell migration. Further studies are needed to elucidate the detailed molecular mechanisms of how NFATc1 can affect the migration of TNBC cells. In addition, in vivo study is needed to confirm the role of TRPC3 in TNBC progression.

The Tunnel hypothesis of urease maturation mechanism

Ka Lung TSANG and Kam Bo WONG

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Speaker

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Urease is a nickel ion-containing metalloenzyme that decomposes urea into ammonia and carbon dioxide. Urease is the first nickel enzyme to be identified as well as the first enzyme to be crystallized, which it also serves as a virulent factor for gastric infection of Helicobacter pylori in human. While nickel ion is essential for urease activity and allows *H. pylori* to survive in the stomach, it is toxic to the cell by actively replacing other weaker metal ions, for example, magnesium ion. To make use of the nickel ion for urease maturation as well as prevent damage to the cells, they developed different accessory proteins to directly deliver the nickel ion to urease and reduce the concentration of free nickel ion in the cytoplasm. In this presentation, we study the interaction of the formation urease activation complex which consists of urease and its accessory protein UreF, UreH(D), and UreG. UreF formed a head-to-head dimer flanked by 2 UreH, resulting in an HFFH pre-activation complex. UreH interacts with the urease, while UreF can recruit UreG dimer and formed the activation complex. The tunnel hypothesis suggested that a nickel delivering tunnel exists in the activation complex, from the interaction site between UreF and UreG dimer, all the way down to the active site of urease. We recently solved the Cryo-EM structure of the Urease-HFFH activation complex of *Helicobacter pylori*. Base on the structure, it shows that binding of *Hp* UreH cause a significant conformational change on *Hp* urease, which open up a tunnel from UreH to the active site of urease. Some key residues were identified from the structure and the mutagenic studies were done. Our mutagenic studies show that the conformational change resulted from urease-UreH(D) interaction is critical for urease activation. A tunnel was reviewed with the recently solved model. From the structure, we found that this tunnel is filled with negatively charged residues and polar residues. Mutagenic study on *Hp* Urease and *Hp* UreH was used to study important residues that contribute to the formation of the nickel delivering tunnel. The study of this nickel delivery tunnel not only let us better understand the maturation mechanism of urease in *H. pylori* but also reveal a strategy of organisms to make use of toxic substances for better adaptation to the environment.

Type 2 innate immune signals regulate endothelial functionality in injured skeletal muscle

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Speaker

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Musculoskeletal condition is the highest contributor to global disability, affecting 20%–33% of people globally. Developing efficient and pro-active therapies to restore functional skeletal muscle for improvement of life quality to patients suffering from musculoskeletal defects is greatly warranted. Angiogenesis is a complex process which requires endothelial cell from pre-existing vessels to migrate and proliferate to form new tube-like structures, thereby maintaining muscle mass and facilitating muscle growth and regeneration after injury. Recent studies have found that innate immunity plays a key role in regulating homeostasis of skeletal muscle tissue. However, how innate immunity regulates endothelial functionality in muscle tissue is still unclear.

Our study aims to examine the mechanisms by which the major drivers of innate immunity, type 2 signals interleukin-4 (IL-4) and its similar type 2 cytokine interleukin-13 (IL-13), contribute to endothelial function and angiogenesis within skeletal muscle tissue.

We hypothesize that IL-4 and IL-13 are required for the overall resolution of skeletal muscle injury by increasing vascularization through IL-4Rα signaling directly on the endothelial cells. We conducted experiments examining the effect of concomitant loss of IL-4 and IL-13 regarding to angiogenesis during skeletal muscle regeneration. Preliminary results indicate defective muscle repair in IL-4/IL-13 double knockout mice (DKO) compared to WT mice. Subsequently, a decrease of endothelial cell populations and total capillary density was also observed in DKO injured muscle. Tube formation studies in mouse endothelial cell line indicate that IL-4 and IL-13 play important roles in regulating endothelial cell angiogenetic capability. Our result has shown IL-4 and IL-13 is involved in angiogenesis during muscle regeneration.

Functional characterization of Arabidopsis RNA cap-modification enzymes DXO1 and CMT

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Speaker

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Eukaryotic mRNAs typically contain the methylguanosine (m^7G) which protects mRNAs from degradation and is involved in all stages of the transcription cycle, nuclear transport, and translation initiation. The DXO family proteins have been known to decap the m^7G cap from mRNAs. Recently, some DXO family proteins in yeast and mammals were found to be able to remove the noncanonical NAD cap. We found that the *Arabidopsis* DXO1 also possesses NAD-RNA decapping activity and 5'-3' exonuclease activity but lacks the m^7G-RNA decapping activity. The *dxo1* mutants displayed pleiotropic phenotypes, including severe growth retardation, pale color, multiple developmental defects, as well as enhanced disease resistance. However, these phenotypes could be genetically complemented by enzymatically inactive DXO1 mutant form, indicating that DXO1 has other functions.

Yeast two-hybrid screen assay was performed to identify DXO1-interacting proteins. An RNA cap methyl transferase (CMT) was identified to interact with DXO1 through DXO1 plant-specific N-terminus. CMT in animals are known to methylate the guanosine cap to form the m7G cap. The function of Arabidopsis CMT has been reported. We found that the *cmt* mutation does not affect gametogenesis but leads to early embryonic lethality. We will further investigate how CMT and DXO1 play roles in mRNA capping and decapping.

*AtMYB*30 is a key regulator in *Arabidopsis* seedlings in response to oxidative stress

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Speaker

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As sessile organisms, plants are continually saddled with miscellaneous abiotic stress. To overcome such predicaments, they have evolved various adaptation mechanisms, and some transcription factors (TFs) act as the molecular basis. To find novel regulators, 79 candidate genes were chosen for this study, fortunately, some promising genes were found. As an example, the KO line of AtMYB30 shows hypersensitivities to salt stress, cadmium stress, and more significantly, to oxidative stress. AtMYB30 was first reported as a key regulator in the biotic stress response network, yet evidence rises that it may also participate in some abiotic stress response and phytohormone signaling pathways. AtMYB30 was reported to regulate the response pathway of oxidative stress, the common secondary stress of biotic and abiotic stresses, by reestablishing the cytosolic calcium homeostasis, but other mechanisms are unveiled.

To find the role of AtMYB30 in abiotic stresses response networks, first, our results indicate that it is crucial in the crosstalk between oxidative stress and NO/ABA signaling pathways. And MIEL1 of the ABA signaling pathway may act as the key node of the crosstalk. Next, some common ROS and antioxidants levels were assayed. The results showed that the KO lines accumulate more O_2^- but H_2O_2 than the OE/WT lines, and the ASA level is significantly lower. DHAR2, a key gene that maintains ASA homeostasis was found to attribute to the differential resistance among the 3 lines. The expression pattern of AtMYB30 was determined, AtMYB30 is expressed mainly in root, cotyledons, and juvenile leaves rather than adult shoot. Further analyses indicate that AtMYB30 may be trans-regulated by MYB7, MYB32, and probably 6 other upstream regulators. This study may give us new insights on the roles of MYB30 in plant abiotic stress regulation networks, and it may give us new clues to solve abiotic stresses faced by agriculturally, economically, and environmentally important plants.

Top-Down Proteomics in Studying Post-Translational Modifications on intact Histones in Plants

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Speaker

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Histone post-translational modifications (PTMs) on nucleosomes alter chromatin accessibility and relevant gene expression in eukaryotes. They are reversely catalyzed by relevant nuclear enzymes, referred to as “writers” (e.g. acetyltransferases) and “erasers” (e.g. deacetylases), which are responsible for adding and removing histone marks, respectively. Histone code, the form or combination of histone PTMs, can be recognized by different “readers”. Histone readers coordinate with other regulatory factors to activate or repress gene transcription for specific biological functions. Previous studies in our laboratory have elucidated that a histone reader GmPHD5 can recognize H3K4me2 and recruits a histone writer GmGNAT1 which catalyzes H3K14ac in soybean (*Glycine max*). We also found that the salinity stress inducible GmPHD5 complex can further activate the expression of salt-responsive genes. Based on these findings, we speculate that there may be crosstalk between diverse histone modifications, either modification on one histone (in cis) or modifications on different histones (in trans). ChIP sequencing of individual histone marks allows us to characterize their distribution at genome-wide level. On the other side, high-resolution mass spectrometry (MS) makes it possible for us to detect all PTMs on each histone. We hypothesis that the combination/ pattern of different PTMs on each histone would change during development or in response to stresses. Conventional “bottom-up” proteomics based on protease (e.g. trypsin) which generate short peptides would limit characterizing various combinations of modifications on full-length histones. In order to overcome this limitation and verify our hypothesis, we aim to develop “top-down” proteomics approaches to characterize the modifications on intact histones. Recently, we have successfully established a modified dual gradient WCX/HILIC (weak cation exchange/hydrophilic interaction LC) to separate histone proteoforms (histones with various combinations of modifications) depending on their acetylation and methylation degree. We plan to adopt this method to perform a comprehensive investigation of histone marks in soybean. Furthermore, we also want to explore the dynamic changes of histone marks’ patterns in response to abiotic stresses by top-down MS. Besides, we will combine ChIP sequencing and transcriptome data to validate the co-currency of histone marks and gene expression. Taken together, these data will provide new insights into histone PTMs in regulating specific biological functions.