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Abstract Booklet

Analysis of soybean acyl-CoA-binding protein expression

Nur Syifaq AZLAN and Mee Len CHYE

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Speaker 1

Acyl-CoA-binding proteins (ACBPs), found in all eukaryotes and some prokaryotes, play important roles in developmental and stress responses. They 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), *Oryza sativa* (rice), and *Brassica napus* (oilseed rape) have revealed that plant ACBPs can be classified according to size and the presence domain(s) adjoining the acyl-CoA-binding domain.

Soybean (*Glycine max*) ACBPs, designated GmACBPs, are not well reported although this legume is a globally important crop cultivated for its high oil and protein content and 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. The subcellular localization of each GmACBP was also predicted and their putative expression profiles in various organs deduced.

Data mining of RNA-sequencing analyses indicated high expression of some Class III GmACBPs in root nodules hinting on their involvement during nodulation, a role not previously encountered for the non-leguminous ACBPs. Root nodules are special organs in legume plants arising from their interaction with the soil bacteria *Rhizobia* in fixing atmospheric nitrogen. Interestingly, the sole member of Class III ACBP in each of non-leguminous Arabidopsis and rice had been previously identified in plant-pathogen interactions. Therefore, studies will focus on identifying the role of GmACBPs in nodulation.

Epigenetic regulation of brassinosteroid signaling in *Arabidopsis*

Guanghua MENG 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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The plant steroid hormones, brassinosteroids (BRs), play important roles in plant development and stress responses. A BR signaling pathway from the membrane receptor kinases BRI1/BAK1, to the nuclear transcription factors BZR1/BES1, has been established. However, the detailed mechanisms as to how BR signaling controls different developmental processes and relevant gene expression have not been fully understood. Some recent studies indicated that certain epigenetic modifications contribute to the regulation of BR signaling. Nevertheless, the evidence for epigenetic regulation of BR signaling remains limited.

In order to identify new epigenetic modifiers involved in BR signaling, we first performed a phenotypic analysis to selected BR mutants under treatment with 5-Aza-2'-deoxycytidine (5-Aza), a DNA methylation inhibitor. Compared with wild type plants, *det2* and *bin2-1*, a BR deficient and signaling mutant, respectively, were severely disrupted in growth by 5-Aza, suggesting that DNA methylation affects BR-mediated plant growth. To identify the possible DNA methylation modifiers involved in BR signaling, we performed a small-scale yeast-two hybrid screen to seek for protein-protein interactions between epigenetic regulators and BR signaling components. Interestingly, an interaction was found between BRASSINOSTEROID-INSENSITIVE 2 (BIN2), a kinase that negatively regulates BR signaling, and METHYLATION ASSOCIATED PROTEIN 1 (MAP1), a histone methylation 'reader' protein that specifically binds to methylated histone 3 lysine 9 (H3K9) and modulates genome-wide DNA methylation. *In vitro* kinase assay indicated that BIN2 can indeed phosphorylate MAP1. The phosphorylation sites of BIN2 on MAP1 were further determined by mass spectrometry and six phosphorylation sites were identified. To understand the function of these phosphorylation, we generated MAP1 phosphorylation- and dephosphorylation-mimicking mutants for MAP1. My preliminary result indicated that the constitutively phosphorylated quadruple mutation of MAP1 eliminated its interaction with BIN2, suggesting that phosphorylation of MAP1 affected its regulation by BIN2. In addition, MAP1 was also found to interact with HDA6, a histone deacetylase. Because histone acetylation and H3K9 methylation are two important epigenetic markers for DNA methylation reprogramming, we believe that the BIN2-MAP1-HDA6 interaction may define a regulatory mechanism of DNA methylation in BR signaling. We will conduct more mechanistic studies to understand how this signaling module modulates BR signaling and plant growth.

Priming-induced alterations in histone modifications modulate transcriptional responses in soybean under salt stress

Wai-Shing YUNG and Hon-Ming LAM

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Speaker

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Plants that have experienced certain abiotic stress may gain tolerance to a similar stress in subsequent exposure. This phenomenon, called priming, was observed in soybean (*Glycine max*) seedlings exposed to salt stress. Time-course transcriptomic profiles revealed distinctively different transcriptional responses in the primed seedlings from those in the non-primed seedlings under high salinity stress, indicating a stress response strategy of repressing unhelpful biotic stress responses and focusing on the promotion of those responses important for salt tolerance. To identify histone marks altered by the priming salinity treatment, a genome-wide profiling of Histone 3 Lysine 4 dimethylation (H3K4me2), Histone 3 Lysine 4 trimethylation (H3K4me3), and Histone 3 Lysine 9 acetylation (H3K9ac) was performed. Our integrative analyses revealed that priming induced drastic alterations in these histone marks, which coordinately modified stress response, ion homeostasis and cell wall modification. Furthermore, transcriptional network analyses unveiled epigenetically modified networks which mediate the strategic downregulation of defense responses. Altering the histone acetylation status using a chemical inhibitor of histone deacetylase could elicit the priming-like transcriptional responses in non-primed seedlings, confirming the importance of histone marks in forming the priming response.

Functional analysis of Prenylated Rab acceptor PRA1.F3 in *Arabidopsis thaliana*

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Speaker

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Members of the Prenylated Rab acceptor 1 (PRA1) family aid the recruitment of prenylated Rabs to their cognate organelles. The *Arabidopsis thaliana* (Arabidopsis) PRA1 family contains a large number of members. Nevertheless, the localization and function of these PRA1 proteins remain largely unknown. Here, we investigated the role of AtPRA1.F3 in endomembrane system.

Our results show that PRA1.F3 transcripts mainly expresses in root tips, vasculatures of roots, true leaves and has high expression in guard cell. GFP-PRA1.F3 was shown to localize to the trans-Golgi, trans-Golgi network (TGN) and tonoplast in both transient and stable transformation systems. Overexpression of AtPRA1.F3 inhibited the trafficking of Aleurain-GFP and Sporamin-GFP to vacuoles and the trafficking of Scamp-RFP and GFP-SYP132 to plasma membrane. However, neither overexpression nor knockout of AtPRA1.F3 affected the trafficking of secretory proteins invertase-GFP, PR1-GFP and Golgi-localized protein ST-GFP. At the cellular level, GFP-AtPRA1.F3 overexpression and *atpra1.f3* knockout plants displayed a highly expanded and vesiculated Golgi apparatus in contrast to the well-stacked Golgi apparatus in wild-type plants.

AtPRA1.F3 also interacts with several Rab proteins (RabA4a, RabA4b, RabG3e) and several SNAREs (VAM3, SYP51 and VAMP713). RabG3e, VAM3, SYP51 and VAMP713 are involved in vacuolar trafficking. Based on these results, we propose that trans-Golgi, trans-Golgi network and tonoplast localized AtPRA1.F3 is involved in the exit of vacuolar proteins to vacuole and membrane proteins to plasma membrane.

Effects of irrigation on regional climate and air quality in northern China

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Graduate Division of Earth and Atmospheric Sciences, Faculty of Science, The Chinese University of Hong Kong

Speaker

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Irrigation, which is designated to boost crop yields by adding extra water to soil, can strongly influence regional climate and air quality by modifying land surface conditions and fluxes. To comprehensively explore the effects of irrigation across northern China, we implemented three irrigation methods (drip, flood and sprinkler) into the Weather Research and Forecast Model (WRF) coupled with the GEOS-Chem (GC) chemical transport model (WRF-GC). Generally, irrigation increases latent heat flux but decreases sensible heat flux, thereby reducing surface temperature and thinning the atmospheric boundary layer in irrigated areas. The magnitudes of the corresponding changes are higher in flood irrigation but similarly lower in drip and sprinkler irrigation, reflecting the importance of irrigation amounts in the three irrigation methods. Notably, irrigation in the pre-monsoon season tends to strengthen the precipitation in “Meiyu” season and shift the rain belt northward to the northern and northeastern China in mid-July to August by causing anomalous anticyclones in North China Plain (NCP) and its surrounding regions. However, the suppressed precipitation in NCP results in higher surface air temperature and lower soil moisture, which indicates the requirement of starting irrigation for crop growth. Through investigating the effects of irrigation on air quality in northwestern China, we also found that the decline in atmospheric boundary layer inhibits vertical mixing of air pollutants and deteriorates surface air quality. Both NO_x and $PM_{2.5}$ increase over irrigated areas. The effects on ozone are complex. This research highlights the effects of irrigation on air quality and the lag effect of spring irrigation on summer monsoon precipitation.

Prediction of foliar functional traits from drone-based hyperspectral imagery

Shuwen LIU and Jin WU

School of Biological Sciences, The University of Hong Kong

Speaker

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Foliar functional traits reflect the strategy of plants in resource acquisition and response to environmental factors, also are widely used to understand and characterize plant properties that drive ecosystem processes. Leaf traits are usually quantified through field measurement, which is labor-intensive and difficult in remote region especially tropical forests. Previous studies have successfully demonstrated that spectroscopy is a useful tool for estimating many types of foliar functional traits. However, to what extent these traits can be derived from drone-based hyperspectral image is still unknown, especially in structure-complicated tropical forests.

Here, we collected drone-based hyperspectral image in a tropical forest and a subtropical forest in China, as well as ground-truth leaf-level traits, including leaf mass per area, leaf water content, chlorophyll content, nitrogen content, phosphorus content, maximum rate of carboxylation ($V_{c,max}$) and electron transport (J_{max}). We will test whether reflectance of individual tree crown from drone hyperspectral image can capture variation of these traits.

Using partial least squares regression, we found that all these traits could be predicted from individual tree crown reflectance. This study exhibits the potential of hyperspectral remote sensing technology in monitoring individual tree-level functional traits in very diverse tropical forests and further evaluating functional diversity.

Techniques for building plant root gene regulatory network

Weilun LIU and Silin ZHONG

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School of Life Sciences, The Chinese University of Hong Kong

Speaker

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Transcription factors play a central role in controlling gene expression. They exert their function and specificity through physically interacting with specific *cis*-regulatory genomic DNA sequences nearby their target genes. One transcription factor may regulate many genes and one gene may be targeted by multiple TFs. Furthermore, TFs themself are also heavily regulated by other TFs. All these forming a gene regulatory network (GRN) underlying the complex gene expression behavior. Plant root has a well organized radially and longitudinally structure and has been used as a model for gene regulatory network construction, analysis and modelling. In the model plant Arabidopsis, several tissue-enriched gene regulatory networks were constructed over the years. These GRNs have proven to be highly useful for gene function inference, uncovering mechanisms behind the expression pattern generation and developmental processes. However, Arabidopsis is the only plant with large scale root GRNs constructed. Other plants such as tomato and maize have root architectures and plasticity that are different from Arabidopsis which need their own root GRNs. High-throughput methods are needed for other plants to build root GRNs that are comparable to the ones in Arabidopsis in a relatively short time. In my research, I will attempt to establish high-throughput and efficient methods for identifying TFs binding site and single-cell omics technology for regulatory interactions inference. Two efficient root transformation methods, hairy root transformation and root protoplasts transfection, were developed facilitating large-scale ChIP-seq experiments. Single-cell RNA-seq and ATAC-seq methods based on combinatorial indexing and droplet microfluidics were also explored. The TF-DNA interactions from ChIP, expression/co-expression patterns from scRNA-seq and cell-type specific chromatin accessibility from scATAC-seq can potentially be integrated to build a comprehensive GRNs. This research would help to expand the research tools that can be utilized in building other GRNs. Also, the data generated for the root TFs and the GRNs constructed would be a valuable resources for future studies.

Characterization of EXPO and EVs in Plants

Jiayang GAO, Yong CUI and Liwen JIANG

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

Speaker

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Extracellular vesicles (EVs) are lipid bound vesicles secreted by cells into the extracellular space. In eukaryotic cells, EVs play important roles in mediating intercellular communication via delivering biomolecules to extracellular space. In mammalian cells, there are three kinds of EVs including 1) exosomes derived from multivesicular bodies (MVB)-plasma membrane (PM) fusion, 2) shedding microvesicles directly budding from the PM, and 3) apoptic bodies generated by cell death.

Distinct from mammalian cells, plant cells contain cell wall which may prevent the formation and function of EVs. Interestingly, recent studies have identified two types of plant EVs: (1) Exosome-like EVs isolated from apoplastic wash of the pathogen-challenged *Arabidopsis* leaves via ultra-centrifugation, and (2) the novel double membrane organelle termed EXPO (exocyst positive organelle) that mediates an unconventional protein secretion (UPS) pathway. However, the nature and identity of EVs in plants *in vivo* remain elusive.

Here we use a combination of 3D Electron Tomography (ET) and Correlated Light microscope and Electron Microscope (CLEM) approaches to study the types and structures of EVs in various plant cell types. Based on their sizes and internal contents, we have classified the identified EVs into three types: Type1 EVs (electron-transparent, 30-200nm in diameter), Type2 EVs (contain ribosomes, 200-500nm in diameter), and Type3 EVs (contain ribosomes and small vesicles, 500-2000nm in diameter) in *Arabidopsis* root cells. Further CLEM and immunogold-TEM analysis have distinguished the Exo70E2-positive EXPO from other types of EVs in term of size, shape and cargo contents in the root apoplast of *Arabidopsis*. Functionally, a new cargo of EXPO has been identified likely to be involved in plant defense against pathogens. On-going studies include functional characterization of EVs in plants. Supported by grants from the NNSF of China (91854201), and RGC of Hong Kong (AoE/M-05/12, C4033-19E, C4002-17G, C4002-20W).

Investigation of the role of *Yin Yang 1* in Mouse Purkinje cell postnatal maturation and dendritogenesis

Ying Lam LUI and Kin Ming KWAN

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Speaker

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Being a well-known zinc-finger transcription factor gene in mammalian development and growth, Yin Yang 1 (YY1) has a dual role in gene expression which can either act as an activator or a repressor by which it consists of inhibitory and activation domain. YY1 becomes many researchers' interests, because it plays a role in cell proliferation regulation, apoptosis, and differentiation via regulating abundant gene expression by disrupting DNA binding transcription factor, or recruiting transcription co-factors, or even modifying DNA conformation. A major consequence of mutated YY1 in human is neurological diseases such as intellectual disability and ataxia. Others have revealed truncated-YY1 in multiple neurodegenerative disorders in human. From our study, conditional inactivation of Yy1 in Purkinje cell (PC) resulted in inhibition of PC dendritogenesis postnatally and ataxia. However, the mechanism underlying the inhibition of PC dendritogenesis caused by Yy1 inactivation remains unclear.

To study the role of Yy1 in PC maturation and dendritogenesis, PC-specific Yy1-conditional knock-out mouse (CKO) model was generated via crossing *Pcp2* (*Purkinje cell protein-2*) - cre with *Yy1* floxed mice. *Pcp2* $^{Cre/+}$; *Yy1*$^{flox/flox}$ and *Pcp2*$^{Cre/+}$; *Yy1*$^{flox/+}$ mice, which are experimental samples and control samples respectively were used in the behavioral testing, immunohistochemisty (IHC), and glogi staining were accomplished to determine the role of *Yy1* in mouse PC dendrite development. From our preliminary results, *Yy1*-CKO mice displayed decreased cerebellum size, shorten PC branches with no density change, and impaired motor balance and coordination. To validate the potential key factor contributing the phenotype of *Yy1*-CKO in PCs, transcriptome profiles of postnatal cerebellum tissue were analysed via next-generation sequencing (NGS). Some potential candidate genes like *Wnt10*, *Fosb* (*FosB Proto-Oncogene*), *Pappa* (*Pappalysin 1*), *Top2A* (*DNA Topoisomerase II Alpha*), *Cd1d*, *IgfbpL1*(*Insulin Like Growth Factor Binding Protein Like 1*) and *Nhlh1* (*Helix-loop-helix protein*) were identified. In this project, we will investigate the novel target of Yy1 regulating PC maturation and dendritogenesis.

Subcellular localization mechanism of SR protein SRSF3

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State Key Laboratory of Agrobiotechnology (CUHK)
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Speaker

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SR proteins (serine/arginine rich proteins) are mRNA splicing factors which characterized by a C terminal serine/arginine rich domain and one or two RNA recognition motifs (RRM) at the N terminus. While most SR proteins are predominately located inside the nucleus, SRSF1, SRSF3 and SRSF7 shuttle between the nucleus and cytoplasm. The localization of SR proteins is critical for their biological functions in the nucleus and cytoplasmic, including the regulation of pre-mRNA splicing, mRNA export and translation.

Transportin SR1 (TRN-SR1) and transportin SR2 (TRN-SR2) can bind specifically and directly to the RS domains of SR proteins. Previous study illustrated that Transportin-SRs facilitate SR proteins import into nucleus and the phosphorylation status of SR proteins is critical for the process.

Our studies on SRSF3, a shuttling SR protein, showed that deletion of the RS domain retained the protein in the cytoplasm. However, when we mutated the serines of the RS domain to alanine, the mutant SRSF3 could still be transported into the nucleus, indicating that while the RS domain is required for the nuclear import of SRSF3, its phosphorylation is not absolutely required. This suggests that SRSF3, in addition to TRN-SR-mediated nuclear import, could enter the nucleus via a phosphorylation independent mechanism. And we hypothesize that there maybe other new karyopherins or transport factors that help to import SRSF3 into the nucleus. My study will focus on the identification of the mechanism that regulate SRSF3 subcellular localization.

Structural insights into how vacuolar sorting receptors recognize the sorting determinants of seed storage proteins

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Speaker

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In *Arabidopsis*, the vacuolar sorting receptor isoform 1 (VSR1) sorts 12S globulins to the protein storage vacuoles during seed development. Vacuolar sorting is mediated by specific protein-protein interactions between VSR1 and the vacuolar sorting determinant located at the C-terminus (ctVSD) on the cargo proteins. Here, we determined the crystal structure of the protease-associated domain of VSR1 (VSR1-PA) in complex with the C-terminal pentapeptide ($_{468}$RVAAA$_{472}$) of cruciferin 1, an isoform of 12S globulins. The $_{468}$RVA$_{470}$ motif forms a parallel β-sheet with the switch III residues ($_{127}$TMD$_{129}$) of VSR1-PA, and the $_{471}$AA$_{472}$ motif docks to a cradle formed by the cargo-binding loop ($_{95}$RGDCYF$_{100}$) making a hydrophobic interaction with Tyr99. The C-terminal carboxyl group of the ctVSD is recognized by forming salt-bridges to the Arg95. The C-terminal sequences of cruciferin 1 and vicilin-like storage protein 22 were sufficient to redirect the secretory red fluorescent protein (spRFP) to the vacuoles in *Arabidopsis* protoplasts. Proline substitutions at the last 4 residues of the ctVSD and R95M substitution of VSR1 disrupted receptor-cargo interactions *in-vitro* and led to increased secretion of spRFP in *Arabidopsis* protoplasts. How VSR1-PA recognizes ctVSDs of other storage proteins were modelled. The last 3 residues of ctVSD prefer hydrophobic residues because they form a hydrophobic cluster with Tyr99 of VSR1-PA. Due to charge-charge interactions, conserved acidic residues, Asp129 and Glu132, at around the cargo-binding site should prefer basic residues over acidic ones in the ctVSD. The structural insights gained may be useful in targeting recombinant proteins to the protein storage vacuoles in seeds.

Investigating the molecular mechanism of plant selective autophagy

Cheuk Ling WUN and Xiaohong ZHUANG

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Speaker

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Autophagy, is a conserved degradation process for eliminating the detrimental components and dysfunctional organelles in cells, which is highly induced upon stress conditions. During this process, a double-membrane compartment called the autophagosome is formed to engulf the harmful materials. Multiple autophagy-related (ATG) proteins and other regulators are recruited for autophagosome formation and cargo recognition. A canonical protein, ATG8, which is conjugated on the autophagosome membrane, has been reported to function throughout the whole autophagy process like membrane expansion, membrane closure and cargo recognition. To sequester specific cargo into the autophagosome, which is termed as selective autophagy, ATG8 binds to the autophagic receptors/ adaptors for the sequestration of cargo into autophagosomes. The majority of known receptors are characterized by an ATG8-interaction motif (AIM) for binding to ATG8. However, the molecular basis for the binding specificity towards ATG8 remains unclear, especially at the molecular level.

Our recent study has uncovered a distinct competition mode in plant selective autophagy, showing that the amounts of receptor proteins may competitively interfere the interaction affinity between ATG8 and a plant-unique adaptor SH3P2. In order to further explore the plant-specific mechanisms in selective autophagy, we have performed a pull-down assay followed by mass spectrometry analysis, and successfully identified several known ATG8 receptors, as well as some novel ATG8-associated candidates. Subcellular analysis showed these candidates are closely associated with ATG8. In future, I will further investigate the underlying mechanism for their interactions with ATG8, as well as their roles in plant selective autophagy.

The proteome of *Arabidopsis thaliana* embryogenesis

Yingzhang HUANG and Dianjing GUO

State Key Laboratory of Agrobiotechnology (CUHK) and

School of Life Sciences, The Chinese University of Hong Kong

Speaker

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In flowering plant, the morphology has established during early embryogenesis. While some of large scale studies focused on transcript level during flowering plant embryogenesis in recent years, the protein level remains largely unexplored. Here, to unveil the proteome dynamics during plant embryogenesis, we performed nano-proteomics using *Arabidopsis thaliana* embryo at 2/4-cell, 8-cell, 16-cell, 32-cell, Globular and Heart stage. In total, 5,387 proteins were identified, with 1051 of them founded in all stages. We founded that there was a dramatically change at protein level either in abundance or newly translated during Globular to Heart stage transition, although transcript level was relatively stable. Unexpectedly, the zygote arrest 1 (ZAR1) showed the highest abundance protein from 2/4-cell to Globular stage, but it decreased sharply at Heart stage, suggesting it may play important roles during early development. Protein-mRNA correlation analysis showed high degree of positive correlation between protein and transcript during Arabidopsis early development. Overall, our highly resolution proteomes provide a valuable resource for plant early developmental study.

Sex-biased genes and responses in Drosophila

Shun Kei TSANG, Yan Nong WEN, Yichun XIE and Ho Lam Jerome HUI

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Speaker

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Male and female organisms often shown morphological, structural and biological differences e.g., muscle mass of human. These sexual dimorphisms are the result of sex-biased genetical controls which experienced years of selections to reach today fitness of each sex and these Gender-related differences in gene expression are extensive across a range of taxa, including plant, nematodes, insects, birds and mammals. Previous studies on sex-biased genes expression were trying to resolve problems like sex-dependent diseases and its implication on evolution.

With the help of model organisms like *Drosophila* species, we are able to find out what are the gene expression differences upon challenging climate and environmental stress. Most of these researches were species specific or with the comparison of different species, population or life stages. However, the regulatory system that drive the sex-differential responses towards various stresses are poorly understood.

In this study, we are going to present differential genes expression in male and female *Drosophila* species under various temperature. We also aim to find out genes that involve in stress responses across different *Drosophila* species and how they are integrated in the overall sex-biased regulation and leaded to sex specific responses / adaptations to environmental stress.

Our preliminary result shows distinct groups of differential gene expression between different sex under each temperature groups. Via mapping those sex-biased genes to different biogenesis pathway, we have visualized pathways that are sensitive to temperature and at the same time expressed differently between two sexes.

We prospect this study can discover correlation of these sex-specific response and potential adaptations to abiotic environmental factor and validate the potential regulation mechanism such as microRNA gene silencing and shine a light on sex-specific environmental adaptions of insects / pests.

Xylem sap peptidomic revealed signaling peptides involved in nitrogen deficiency response in soybean

Wai Ching SIN and Sai Ming NGAI

State Key Laboratory of Agrobiotechnology (CUHK) and

School of Life Sciences, The Chinese University of Hong Kong

Speaker

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The transportation of hormones in plants play vital roles in maintaining their development and resistance to stresses. Secreted peptide hormones are typically post translational modified oligopeptides that are cleaved from precursor proteins and subsequently secreted extracellularly. There is increasing evidence suggesting that secreted peptides transported via xylem sap can coordinate root-to-shoot communication to modulate plant growth and response against abiotic and biotic stresses.

In our study, we developed a LC-MS workflow to analyse the secreted peptidome of soybean xylem sap exudate. From the MS spectra, we successfully identified 18 peptide hormone candidates from soybean xylem sap exudate that correspond to the secreted product of plant peptide hormones. Moreover, most candidate were identified with conserved plant hormone PTMs, including tyrosine sulfation, hydroxylation or tri-arabinosylation on proline. Quantitative PCR analysis revealed peptide precursor genes are expressed in multiple tissues including some genes that are root specific. Further qPCR analysis showed that some peptide genes show distinctive respond to abiotic stress factors, including drought stress, nitrogen deficiency and iron deficiency. We further studied on two CEPs (C-TERMINALLY ENCODED PEPTIDE) that are significantly up-regulated during nitrogen deficiency treatment.

To quantify the peptide abundance level in xylem sap exudate under stress, we applied targeted MS quantification method. We successfully demonstrated the increased secretion of peptide hormones in soybean xylem sap during low nitrogen stress. We further attempted to study the functional roles of low nitrogen stress responsive CEPs via proteomic analysis. From proteome profiles of *Agrobacterium* induced hairy roots, the overexpression of CEPs was found to induce differentially expressed proteins (DEPs) related to stress response and anatomical development. These results provide hints to the still under-explored root-to-shoot signaling network in plant for understanding plant-environment interaction.

Role of Mitochondria in Regulating Apoptosis Reversal in Breast Cancer

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Speaker

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Cancer is one of the top killers in developed countries. Thanks to the recent research on cancer therapy, including immunotherapy and targeted therapy, the survival rates of combined cancer therapy on breast cancer have increased. However, cancer relapse is still the main issue in the eradication of cancer. Previous studies suggested that cancer stem cells (CSCs) are responsible for the emergence of more invasive, therapeutic-resistant tumours.

A recent study from our laboratory has shown that percentage of CSCs was increased after the withdrawal of drug treatment in breast cancer cells, and that some epigenetics changes of CSC markers such CD24 and CD44 were detected. Targeting CSCs can be a novel strategy to decrease the occurrence of cancer relapse in breast cancer, but the underlying mechanism of the appearance of CSCs during apoptosis reversal in breast cancer is unclear.

In our current study, we aimed to investigate if mitochondrial biogenesis and / or dynamics would change in breast cancer cells after apoptosis reversal, and if yes, whether the changes are responsible for the increased aggressiveness of the cells. By Sequential Window Acquisition of all THeoretical fragment ion spectra Mass Spectrometry (SWATH-MS) on apoptosis reversed cells, proteins involved in mitochondrial fusion were found to be upregulated. By MitoTracker staining followed by confocal microscopy, mitochondrial biogenesis was found to increase while mitochondrial dynamics was found to shift towards fusion. To further investigate the role of mitochondrial biogenesis and dynamics in regulating apoptosis reversal in breast cancer, the expression of genes regulating mitochondrial biogenesis and dynamics will be studied. Besides, the possible relationship between CSC cell surface markers and the expression of genes related to mitochondrial biogenesis and dynamics will be characterized. In addition, the effect of mitochondrial biogenesis inhibition on the appearance of CSCs and cancer progression after apoptosis reversal in breast cancer will be studied. This study would provide insights into the possible strategies for future therapeutic treatment of breast cancer.

Endothelial Leptin Resistance Exacerbates Vascular Dysfunction through Hindering PD-L1 Expression

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Speaker

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Obesity is becoming a global health challenge as more people adopt urbanized lifestyles with decreased energy expenditure, and it is closely linked to comorbidities of cardiovascular diseases, the leading cause of death worldwide. Obesity-related cardiometabolic risk factors, such as hyperglycemia and hyperlipidemia, predispose vascular endothelium towards dysfunctionality which is characterized by increased inflammatory responses and decreased bioavailability of vasodilator nitric oxide (NO). Leptin, a peptide adipokine primarily from white adipose tissue, is considered as risk factor associated with obesity. Engagement of leptin to its receptor sends a satiety signal to hypothalamic neurons, thereby suppressing appetite. When there is excessive fat accumulation in obesity, hyperleptinemia appears and may induce selective leptin resistance in several cells including endothelial cells (ECs). Of note, whether endothelial leptin resistance impairs vascular function has yet to be explored. Furthermore, in response to inflammatory challenge, ECs will express programmed cell death ligand 1 (PD-L1; encoded by gene *Cd274*) to provide a suppressive signal to effector lymphocytes, preventing an exaggerated immunogenic attack. Despite extensive research of PD-L1 in cancer biology, its role in metabolic syndrome and CVDs remains largely unknown. In current study, we suggested although leptin is a promising regulator of endothelial PD-L1 expression, chronic hyperleptinemic stimuli induces endothelial leptin resistance that hinder PD-L1 expression; meanwhile, we highlight the intrinsic importance of endothelial PD-L1 expression in regulating inflammatory responses and NO production.

By employing several animal models, we demonstrated preservation of leptin signaling was crucial in endothelial PD-L1 expression, which was also positively correlated with serum leptin levels. Although diet-induced obesity resulted in hyperleptinemia, it was interesting that there was no heightened PD-L1 expression in aortas from C57BL/6 and *Apoe*$^{-/-}$ mice after feeding with long-term high fat diet. Our findings suggested that an increase in negative regulators of leptin signaling may play a role in desensitizing leptin signaling, such that aortic ECs failed to respond leptin stimuli in expressing PD-L1. More importantly, our present study demonstrated how endothelial PD-L1 regulated intrinsic functions under inflammation, particularly inflammatory responses, NO production and vascular reactivity, indicating the significance of endothelial PD-L1 expression as a signaling receptor in preserving endothelial functionality.

Characterization of NAD-capped RNAs using NAD tagSeq II and a high-accuracy method in *E. coli* and *Arabidopsis thaliana*

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Speaker

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Recent findings regarding NAD^+-capped RNAs (NAD-RNAs) indicate that prokaryotes and eukaryotes employ non-canonical RNA capping to regulate gene expression. Two methods for transcriptome-wide analysis of NAD-RNAs, NAD captureSeq and NAD tagSeq, are based on copper-catalyzed azide-alkyne cycloaddition (CuAAC) click chemistry to label NAD-RNAs. However, copper ions can fragment/degrade RNA, interfering with the analyses.

We have reported development of NAD tagSeq II, which uses copper-free, strain-promoted azide-alkyne cycloaddition (SPAAC) for labeling NAD-RNAs, followed by identification of tagged RNA by single-molecule direct RNA sequencing. We used this method to compare NAD-RNA and total transcript profiles of *E. coli* cells in the exponential and stationary phases. We identified hundreds of NAD-RNA species in *E. coli* and revealed genome-wide alterations of NAD-RNA profiles in the different growth phases. Most highly expressed genes produced no or few NAD-RNA, whereas the transcripts of some genes were found to be primarily NAD-capped.

The use of SPAAC click chemistry in place of CuAAC circumvents RNA degradation. However, NAD tagSeq II has limitations such as (i) the difficulty in correctly basecalling the 5’ ends of NAD-RNAs and (ii) the inability to identify some NAD-RNAs smaller than ~100 nt, and (iii) the inefficiency in identification of low-abundance NAD-RNAs. To address the above limitations, we recently developed a high-accuracy NAD Seq, named precision NAD-Seq (or NAD-pSeq). Using NAD-pSeq, we profiled NAD-RNAs in *E. coli* and *Arabidopsis*. We not only mapped the 5’ ends of NAD-RNAs at single nucleotide resolution, but also identified some low abundance NAD-RNAs and small NAD-RNAs.

Structural variation studies in plants and graph genomes

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Genome structural variations (SVs) are important genomic features, which may play important roles in phenotypic variations by deleting, inserting, inverting, or duplicating particular genes. In previous studies, due to technical limitations, SVs were difficult to decipher. With recent advances in DNA sequencing and whole-genome mapping, SV studies are increasing. In our recent soybean research, we have detected some large and complex soybean SVs, which may relate to important agronomic traits, such as seed coat and trailing growth. These findings could be useful for soybean breeding.

With more individuals being studied in a species, a pangenome has been built to capture the core genome and variable genome. However, the normally used linear genome reference cannot represent the full genomic features in a species, making the exploration of the species difficult. To solve this problem, graph genomes, as alternatives, have been proposed. However, there are fewer tools to help check pangenome graphs. In our recent study, we have developed a graph genome viewer to help check the genomic features in a species, therefore, increasing the comprehensive understanding of a pangenome.