The 90th anniversary celebration of National Taiwan University
NTU Hub— College of Life Science

「International Symposium on Plant and Environment Interaction」

December 7 (Friday), 2018, NTU, Taipei, Taiwan

Organizations:

Institute of Plant Biology
College of Life Science
College of Bio-Resources and Agriculture

Organizing Committee:

Shih-Tong Jeng, Dean and Professor, College of Life Science, Institute of Plant Biology
Huu-Sheng Lur, Dean and Professor, College of Bio-Resources and Agriculture, Department of Agronomy
Hsu-Liang Hsieh, Director and Professor, College of Life Science, Institute of Plant Biology
Ke-Qiang Wu, Distinguished Professor, College of Life Science, Institute of Plant Biology
Tsung-Luo Jinn, Professor, College of Life Science, Institute of Plant Biology
Chiu-Ping Cheng, Professor, College of Life Science, Institute of Plant Biology
Yi-Sheng Cheng, Professor, College of Life Science, Department of Life Science and Institute of Plant Biology
Shu-Jen Wang, Professor, College of Bio-Resources and Agriculture, Department of Agronomy

General Meeting Information

Registration area hours

On-site registration, pickup of the abstract book for those who have pre-registered can be done at the Registration Area, located at the 3F of Life Science Building, National Taiwan University.

Special service

If you require special assistance or service, please let a conference staff know at the registration desk.

Coffee Breaks and refreshment

Complimentary coffee and refreshment will be available in the Rm 327. Drinking water (hot/cold) is available on the hall way close to Rm 327.

Catering

Lunches will be provided to registered participants during the conference. You can have your lunch at Rm 327 and 3A classroom. No food is allowed in the conference room (Rm 332).

Smoking

Please note that it is against the law to smoke in any indoor venues of NTU, and on campus of NTU.

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Agenda

Time	Title	Speaker
8:00-8:30	Registration	
8:30-8:40	**Opening remarks**	Dean Shih-Tong Jeng Dean Huu-Sheng Lur
I. Plant Growth and Development (Chair: Hsu-Liang Hsieh)		
8:40-9:10	Molecular mechanisms underlying thermal adaptation of the circadian clock	Prof. Chung-Mo Park
9:10-9:40	To translate or not? Distinct fates of mRNAs in skotomorphogenic and photomorphogenic Arabidopsis seedlings	Director Shu-Hsing Wu
9:40-10:10	Molecular and genetic analysis of anaerobic germination under submergence in rice (*Oryza sativa*)	Prof. Chih-Wei Tung
10:10-10:40	Reciprocal cross-regulation of VND and SND multigene TF families for wood formation in *Populus trichocarpa*	Prof. Ying-Chung Lin
10:40-11:00	**Break & Group Photo**	
II. Plant Epigenetics and Gene Regulation (Chair: Ke-Qiang Wu)		
11:00-11:30	Study of plant cell signaling using the 4C quantitative PTM proteomics	Prof. Ning Li
11:30-12:00	Genome wide DNA methylation in plants	Dr. Pao-Yang Chen
12:00-12:30	Plant autophagy involves in HC-Pro-mediated gene silencing suppression	Prof. Shih-Shun Lin
12:30-13:00	Structural and functional analyses reveal Histone Deacetylase 15 regulated by oligomerization and phosphorylation in Arabidopsis	Prof. Yi-Sheng Cheng
13:00-14:00	**Lunch**	

III. Plant-Microbe Interactions (Chair: Shu-Yi Yang &/or Chiu-Ping Cheng)		
14:00-14:30	Quorum sensing of *Ralstonia solanacearum*	Prof. Yasufumi Hikichi
14:30-15:00	Genetic and environmental factors regulating *Agrobacterium*-mediated plant transformation	Dr. Erh-Min Lai
15:00-15:30	Functional characterization of *Medicago truncatula* phosphate transporter 4 in arbuscular mycorrhizal symbiosis	Prof. Wei-Yi Lin
15:30-16:00	Modulators of the *Arabidopsis thaliana* innate immunity response	Prof. Laurent Zimmerli
16:00-16:20	**Break**	
IV. Plant Abiotic Stresses and Hormones (Chair: Tsung-Luo Jinn)		
16:20-16:50	BAK1, a key regulator in plant development and immunity	Prof. Jia Li
16:50-17:20	Try to remember: regulation of heat stress memory in plants	Dr. Yee-Yung Charng
17:20-17:50	Effect of LiCl on salt stress response in *Arabidopsis thaliana*	Prof. Ing-Feng Chang
17:50-18:20	Role of the rice transcription factors OsbHLH068 and OsbHLH035 in flowering and salinity stress	Prof. Men-Chi Chang
18:20-18:30	**Closing remarks**	Director Hsu-Liang Hsieh
19:00-	**Banquet**	

Abstracts

Molecular mechanisms underlying thermal adaptation of the circadian clock

Chung-Mo Park
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Plant growth and development is widely affected by diverse temperature conditions. While studies have been focused mainly on the effects of stressful temperature extremes in recent decades, nonstressful ambient temperatures also influence a wide array of plant growth and morphogenic aspects, a process termed thermomorphogenesis. Notably, accumulating evidence indicate that both stressful and nonstressful temperatures modulate the functional process of the circadian clock, a molecular timer of biological rhythms in higher eukaryotes and photosynthetic prokaryotes. It is known that the circadian clock can sustain robust and precise timing over a range of physiological temperatures. Genes and molecular mechanisms governing the temperature compensation process have been explored in different plant species. In addition, a ZEITLUPE/HSP90-mediated protein quality control mechanism helps plants maintain the thermal stability of the clock by removing insoluble aggregates of cellular proteins under heat stress. Furthermore, a CCA1-mediated self-regulatory circuit, which is consisted of cold temperature-responsive alternatively spliced CCA1 protein splice variants, contributes to freezing tolerance by sacrificing the circadian rhythms. The thermal adaptation capability and plasticity of the clock are of particular interest in view of the growing concern about global climate changes. I will present our recent data expanding our knowledge of temperature regulation of the clock function in plants. I will also discuss stimulating ideas on the thermal control of the circadian clock along with ecosystem management and future agricultural innovation.

References

1. Seo PJ, Park MJ, Lim MH, Kim SG, Lee M, Baldwin IT, Park CM (2012). A self-regulatory circuit of CIRCADIAN CLOCK-ASSOCIATED1 underlies the circadian clock regulation of temperature responses in Arabidopsis. **Plant Cell** 24: 2427-2442.
2. Gil KE, Kim WY, Lee HJ, Faisal M, Saquib Q, Alatar AA, Park CM (2017). ZEITLUPE contributes to a thermoresponsive protein quality control system in Arabidopsis. **Plant Cell** 29: 2882-2894.
3. Gil KE, Park CM (2018). Thermal adaptation and plasticity of the plant circadian clock. N**ew Phytologist (online publication).**

To translate or not? Distinct fates of mRNAs in skotomorphogenic and photomorphogenic Arabidopsis seedlings

Guan-Hong Chen, Geng-Jen Jang, Shu-Hsing Wu
Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

Skotomorphogenesis and photomorphogenesis are both crucial developmental processes to ensure the survival advantages of a young plant seedling. Light signals initiate the transition from skotomorphogenesis to photomorphogenesis by triggering massive reprogramming of transcriptome and translatome. The comparison of transcriptome and translatome in dark-grown and de-etiolating seedlings revealed about 1,500 mRNAs that have comparable abundance before and after light treatment, yet their translation increases after light illumination. This implies these mRNAs are translationally repressed in dark-grown seedlings. Compared to the wealth knowledge of transcriptional regulation, the molecular mechanism underlying the prominently enhanced translation by light signals remains unclear. Our study suggested the light-enhanced translation relies on two highly coordinated strategies Arabidopsis seedlings use under dark or light conditions. When germinated in the dark, translation of many mRNAs is stalled by the repression of target of rapamycin (TOR)-ribosomal protein S6 (RPS6) phosphorelay and also by temporarily storing pre-existing mRNAs in processing bodies, a class of RNA granules. Upon light treatment, TOR-dependent phosphorylation of RPS6 is activated, accompanied by the reduction of processing bodies to release mRNAs for translation. This study provides mechanistic views and the biological significance of translational regulation to warrant the growth fitness of Arabidopsis seedlings.

Molecular and genetic analysis of anaerobic germination under submergence in rice (*Oryza sativa*)

Sheng-Kai Hsu and Chih-Wei Tung
Department of Agronomy, National Taiwan University, Taipei, Taiwan

Diverse rice accessions and recombinant inbred lines (RILs) were screened for their responses to submergence at germination stage. Differential anaerobic response was observed between *Japonica* and *Indica* subgroups. Many SNPs identified from our genome-wide association studies GWAS were associated with anaerobic germination, some genomic regions were also detected in previous bi-parental QTL studies. A unique and strong signal explaining ~30% of the phenotypic variation was only detected in our RILs population. Our study demonstrated linkage mapping and GWAS are complementary for dissecting the genetic architecture of complex traits in rice. By applying comparative transcriptomic analysis using six rice genotypes with different tolerance to submergence, we identified a core set of genes being differentially regulated under submergence, these differential modulations could contribute to the fundamental tolerance across rice genotypes. Allele-specific gene regulations and g enotype-specific adaptive mechanisms could further enhance the submergence tolerance in the genotypes with extreme tolerant phenotype. Through genetic and comparative transcriptomic analysis, our results provide a comprehensive overview on how different rice genotypes germinate and survive under the oxygen/energy deficit induced by submergence, we expect to apply these information to improve the submergence tolerance in modern rice cultivars.

References

Sheng-Kai Hsu and Chih-Wei Tung* (2017) RNA-seq analysis of diverse rice genotypes to identify the genes controlling coleoptile growth during submerged germination. **Front. Plant Sci.** 8:762 (*Corresponding author)

Sheng-Kai Hsu and Chih-Wei Tung* (2016) Genetic mapping of anaerobic germination-associated QTLs controlling coleoptile elongation in rice**.** **RICE** 8:38 (*Corresponding author)

Reciprocal cross-regulation of VND and SND multigene TF families for wood formation in *Populus trichocarpa*

Ying-Chung Jimmy Lin[a,b,c,d], Hao Chen[d], Quanzi Li[e], Wei Li[a,d], Jack P. Wang[a,d], Rui Shi[f], Sermsawat Tunlaya-Anukit[d], Peng Shuai[d,g], Zhifeng Wang[a], Hongyan Ma[a], Huiyu Li[a,d], Ying-Hsuan Sun[h], Ronald R. Sederoff[d], and Vincent L. Chiang[a,d]

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Secondary cell wall (SCW) biosynthesis is the biological process that generates wood, an important renewable feedstock for materials and energy. NAC domain transcription factors, particularly Vascular-Related NAC-Domain (VND) and Secondary Wall-Associated NAC Domain (SND) proteins, are known to regulate SCW differentiation. The regulation of VND and SND is important to maintain homeostasis for plants to avoid abnormal growth and development. We previously identified a splice variant, PtrSND1-A2^{IR}, derived from PtrSND1-A2 as a dominant-negative regulator, which suppresses the transactivation of all PtrSND1 family members. PtrSND1-A2^{IR} also suppresses the self-activation of the PtrSND1 family members except for its cognate transcription factor, PtrSND1-A2, suggesting the existence of an unknown factor needed to regulate PtrSND1-A2. Here, a splice variant, PtrVND6-C1^{IR}, derived from PtrVND6-C1 was discovered that suppresses the protein functions of all PtrVND6 family members. PtrVND6-C1^{IR} also suppresses the expression of all PtrSND1 members, including PtrSND1-A2, demonstrating that PtrVND6-C1^{IR} is the previously unidentified regulator of PtrSND1-A2. We also found that PtrVND6-C1^{IR} cannot suppress the expression of its cognate transcription factor, PtrVND6-C1. PtrVND6-C1 is suppressed by PtrSND1-A2^{IR}. Both PtrVND6-C1^{IR} and PtrSND1-A2^{IR} cannot suppress their cognate transcription factors but can suppress all members of the other family. The results indicate that the splice variants from the PtrVND6 and PtrSND1 family may exert reciprocal cross-regulation for complete transcriptional regulation of these two families in wood formation. This reciprocal cross-regulation between families suggests a general mechanism among NAC domain proteins and likely other transcription factors, where intron retained splice variants provide an additional level of regulation.

References: *Proc. Natl. Acad. Sci. USA*. 2017, 114(45): E9722-E9729.

Study of plant cell signaling using the 4C quantitative PTM proteomics

Ning Li
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Hong Kong SAR, China

Quantitative and functional post-translational modification (PTM) proteomics has emerged as one of the powerful Omics approaches in the study of cellular events in various model organisms. In this seminar, I intend to show several examples on how to apply *in planta* metabolic labeling- and *in vitro* chemical labeling-based quantitative PTM proteomics workflow (*SILIA, SQUA-D and AQUIP*) in investigation of cell signaling in the model plant Arabidopsis and explain its potential impact in the field of plant cell biology research in general. To elucidate the molecular mechanism underlying plant cell signaling on a number of Arabidopsis responses, several well-known Arabidopsis loss-of-function mutants (***ctr1-1, rcn1-1, etr1-6, ein2-5*** and ***eil3eil1***) of ethylene response were selected as the target plant materials for the PTM quantitation. The 4C quantitative PTM proteomics results clearly revealed that there exist multiple PTM-mediated ethylene signaling in Arabidopsis. This *SIML*-based quantitative PTM proteomics was able to identify rapidly phosphorylated proteins in response either to seconds of touch signal or minutes of hormone or gravity stimulation in Arabidopsis. The biological functions of these key candidate phosphoproteins in these internal and external signals-mediated cellular events were confirmed and validated *via* the follow-up reverse genetics, molecular and cellular biology approaches. These successful application suggests that the quantitative, repeatable, accurate and versatile PTM proteomics can help address many important questions in life sciences.

Representative Publications

1. Wang, K., Yang, Z., Qing, D., Ren, F., Liu, S., Zheng, Q., Liu, J., Zhang, W., Dai, C., Wu, M., Chehab, E.W., Braam, J.,**Li, Ning***. (2018). Quantitative and functional posttranslational modification proteomics reveals that TREPH1 plays a role in plant touch-delayed bolting. **Proceedings of the National Academy of Sciences.** 115, E10265–E10274.
2. Liu, SC, Yu, FC, Yang Z, Wang T, Xiong, H Chang, C, Yu, WC*, and **Ning Li*** (2018) Establishment of dimethyl labelling-based quantitative acetylproteomics in Arabidopsis. **Mol Cell Proteomics** 17, 1010 -1027.
3. Qing, Dongjin, Yang, Zhu, Li, Mingzhe, Wong, Wai Shing, Guo, Guangyu, Liu, Shichang, Guo, Hongwei, **Ning Li***(2016) Quantitative and Functional Phosphoproteomic Analysis Reveals that Ethylene Regulates Water Transport via the C-terminal Phosphorylation of Aquaporin PIP2;1 in Arabidopsis. **Molecular Plant**. 9, 158–174.
4. Zhu Yang, Guangyu Guo, Manyu Zhang, Claire Y. Liu, Qin Hu, Henry Lam,Han Cheng, Yu Xue, Jiayang Li, and **Ning Li***(2013) Stable Isotope Metabolic Labeling-Based Quantitative

Phosphoproteomic Analysis of Arabidopsis Mutants Reveals Ethylene-Regulated Time-Dependent Phosphoproteins and Putative Substrates of CONSTITUTIVE TRIPLE RESPONSE 1 Kinase. **Mol Cell Proteomics**. 12, 3559-3582.

5. Li Y, Shu Y, Peng C, Zhu L, Guo G, **Li, Ning*** (2012) AQUIP: Absolute Quantitation of Isoforms of Post-translationally modified proteins in transgenic organism. **Mol Cell Proteomics**. 2012 11: 272-285.

Genome wide DNA methylation in plants

Pao-Yang Chen
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DNA methylation is an important epigenetic modification involved in many biological processes. Bisulfite treatment coupled with high-throughput sequencing (BS-seq) provides a practical approach to profile genome-wide DNA methylation at base resolution. The focus of our research is on the development of both experimental and computational methods to interpret genomic and epigenomic data from plants, animals and human. By integrating *-omic* data produced from the next-generation sequencing technologies, we devise novel analytical strategies to answer biological questions. In this talk, I will give an quick overview of our profiling pipeline for the epigenomic data analysis, covering both experimental and computational methods. I will also present case studies with integrative (epi)genomic analyses in rice, maize, and Arabidopsis.

References

Fei-Man Hsu, Moloya Gohain, Archana Allishe, Yan-Jiun Huang, Jo-Ling Liao, Lin-Yun Kuang and **Pao-Yang Chen** (2018) Dynamics of the Methylome and Transcriptome during the Regeneration of Rice. **Epigenomes** 2(3), 14

Yu Yuan Huang, Yan-Jiun Huang, **Pao-Yang Chen** (2018) BS-Seeker3: Ultrafast pipeline for bisulfite sequencing. **BMC Bioinformatics** 19:111

Fei-Man Hsu, Chung-Ju Rachel Wang* and **Pao-Yang Chen*** (2018) Reduced Representation Bisulfite Sequencing in Maize. **Bio-protocol** 8(6): e2778. DOI: 10.21769/BioProtoc.2778

Ming-Ren Yen, Der-Fen Suen, Fei-Man Hsu, Yi-Hsiu Tsai, Hongyong Fu, Wolfgang Schmidt*, and **Pao-Yang Chen*** (2017) Deubiquitinating enzyme OTU5 contributes to DNA methylation patterns and is critical for phosphate nutrition signals. **Plant Physiology** 175 (4) 1826-1838

Plant autophagy involves in HC-Pro-mediated gene silencing suppression

Shih-Shun Lin
Institute of Biotechnology, National Taiwan University

HC-Pro is a viral suppressor that suppresses gene silencing in viral infected plants. HC-Pro physical binds HEN1 methyltransferase, resulting in inhibition of microRNA (miRNA) methylation. The FRNK motif of HC-Pro (HC^R) plays a role in HEN1 interaction, whereas the FKNK mutant of HC-Pro (HC^K) lost the interaction with HEN1. Moreover, the *in vitro* assay indicated that HC^R inhibits HEN1 methyltransferase activity through the inhibition of the HEN1-miRNA duplex binding, resulting in unmethylated miRNAs (unMet-miRNA). These unMet-miRNAs cannot be loaded into AGO1, but accumulated in the cytoplasm as a free form. The AGO1 protein is degraded in transgenic Arabidopsis expressing *HC-Pro^R* gene (*HC^R* plant) through the autophagy pathway. By contrast, the HC^R cannot trigger AGO1 autophagic degradation in autophagy mutants, suggesting HC^R can trigger AGO1 autophagic degradation. Interestingly, the unMet-miRNA can be detected in AGO1 in the autophagy mutant background, suggesting autophagy might play as a surveillance system to monitor the status of AGO1-miRNA complex. We assume that unMet-miRNA-AGO1 complex might trigger autophagy for degradation to make sure only methylated miRNAs (Met-miRNAs) loading into AGO1. We hypnotize that HC^R inhibits HEN1 activity on miRNA methylation, resulting in unMet-miRNAs for an abnormal AGO1-miRNA complex to trigger AGO1 autophagic degradation.

Structural and functional analyses reveal Histone Deacetylase 15 regulated by oligomerization and phosphorylation in Arabidopsis

Yi-Sheng Cheng
Institute of Plant Biology, Department of Life Science, and Genome and Systems Biology Degree Program, National Taiwan University

Histone acetylation catalyzed by histone acetyltransferases and deacetylation by histone deacetylases (HDACs) are important post-translational modifications for nucleosome. HDACs catalyze the removal of acetyl groups from lysine residues of histones. Mammalian HDACs undergo phosphorylation to regulate their localization, activity and function, however, little is known about plant HDAC phosphorylation. Here, we reported that the structure and function of the RPD3/HDA1 type class II histone deacetylase HDA15 and showed the activity of HDA15 is negatively regulated by phosphorylation in Arabidopsis. The N-terminal zinc finger domain (ZF) and C-terminal nuclear export signal (NES) are important for the activity of HDA15. The ZF enhances the dimerization and activity of the histone deacetylase domain (HD) of HDA15 *in vitro*. Crystal structure of HDA15 HD showed that the HD assembles as tetrameric form and each monomer is composed of 12 α-helices and 9 β-sheets. LC-MS/MS analysis identified two important phosphorylation sites of HDA15 at Ser 448 and Ser 452, which are conserved in Arabidopsis HDA5, HDA14 and HDA18 as well as human HDAC6 and HDAC10. Phosphomimetics of HDA15 HD containing the zinc finger domain (ZFHD) results in monomerization and loss of HDAC activity. Furthermore, phosphomimetics of HDA15 disrupted its oligomerization and results in translocation from the nucleolus into nucleoplasm *in vivo*. Together, these data indicated that phosphorylation plays a critical role in regulation the structure and function of HDA15 in Arabidopsis.

Quorum sensing of *Ralstonia solanacearum*

Yasufumi Hikichi
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The soil-borne plant pathogenic bacterium *Ralstonia solanacearum* colonizes intercellular spaces after invading roots and then invades xylem vessels, where its high level of multiplication leads to wilting symptoms because the large bacterial population and exopolysaccharide slime produced by the bacteria reduce sap flow (1).

On the other hands, I have focused colonization of intercellular spaces by *R. solanacearum* strain OE1-1 to elucidate its virulence mechanism (1, 2). After invading the intercellular spaces of tomato plants, cells of strain OE1-1 formed mushroom-shaped biofilms (mBFs) on the surfaces of tomato cells, which is involved in virulence of the strain OE1-1 (3). The vigorous growth of *R. solanacearum* during mBF formation led to quorum sensing (*phc* QS), which is required for its virulence. In my talk, I focus the *phc* QS mechanism.

The *R. solanacearum* strain OE1-1 produced methyl 3-hydroxymyristate (3-OH MAME) as quorum sensing signal (4). 3-OH MAME was synthesized by the methyltransferase PhcB. When 3-OH MAME reaches a threshold level, it enhanced the ability of the histidine kinase PhcS to activate the *phc* QS, resulting in elevated levels of functional PhcA, a LysR-type transcriptional regulator, which plays a central role as the global virulence regulator.

R. solanacearum synthesizes aryl-furanone secondary metabolites known as ralfuranones A, B, I, J, K and L (5, 6). The expression of ralfuranone synthase, encoded by *ralA*, is dependent on PhcA activated through *phc* QS. Ralfuranones are involved in both mBF formation (7) and the feedback loop of *phc* QS regulation (8). It is thus thought that the *phc* QS-dependent ralfuranones are involved in intercellular communication during mBF formation and *phc* QS.

When *R. solanacearum* invades the intercellular spaces of plant roots, the expression of *lecM*, encoding the lectin LecM, was induced by the *hrp* regulon's transcriptional regulator HrpG (3). LecM is involved in the attachment of OE1-1 cells to the surfaces of plant cells after invasion into intercellular spaces, thereby contributing to mBF formation. Furthermore, LecM production is also induced by PhcA activated through *phc* QS in *R. solanacearum*. This QS-dependent LecM affects the activation of *phc* QS through the instability of extracellularly secreted 3-OH MAME, thereby contributing to the *phc* QS signaling pathway (9).

Overall, the *phc* QS-dependently produced compounds, not only ralfuranones but also LecM, may synergistically contribute to positive feedback of *phc* QS, and are implicated in the elaborate and tunable regulation of *R. solanacearum* virulence.

References

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   DOI: 10.1080/09168451.2015.1116931.
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9. Hayashi, K., Hikichi, Y. *et al*. 2018. Mol. Plant Pathol., **in press**. **DOI:** 10.1111/MPP.12757.

Genetic and environmental factors regulating *Agrobacterium*-mediated plant transformation

Erh-Min Lai
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Agrobacterium tumefaciens is the causal agent of crown gall disease in a wide range of plants via a unique interkingdom DNA transfer from bacterial cells into the plant genome. *A. tumefaciens* is capable of transferring its T-DNA into different plant parts at different developmental stages for transient and stable transformation. However, the plant genes and environmental factors involved in these transformation processes are not well understood. By transcriptome analysis, several genes involved in indole glucosinolate (iGS) modification and camalexin biosynthesis pathway were up-regulated while genes in aliphatic GS (aGS) biosynthesis were generally down-regulated upon *Agrobacterium* infection. Thus, we evaluated the impacts of GSs and camalexin during different stages of *Agrobacterium*-mediated transformation combining *Arabidopsis* mutant studies, metabolite profiling, and exogenous applications of various GS hydrolysis products or camalexin. The results suggest that the iGS hydrolysis pathway plays an inhibitory role in transformation efficiency on *Arabidopsis* seedlings at the early infection stage. Later in the *Agrobacterium* infection process, accumulation of camalexin was a key factor inhibiting tumor development on *Arabidopsis* inflorescence stalks. In view of the importance of *Agrobacterium*-mediated transient expression as a powerful analysis platform for diverse plant gene functional studies, we further developed a highly efficient and robust *Agrobacterium*-mediated transient expression system, named AGROBEST (*Agrobacterium*-mediated enhanced seedling transformation) in *Arabidopsis* seedlings. we found that AGROBEST was able to promote the growth of agrobacteria as well as inhibit the host immunity response. When the factor of agrobacterial growth is minimized, we discovered that maintaining pH at 5.5 with MES buffer was the key to achieve the optimal transient expression efficiency. Plant immunity marker genes, *FRK1* and *NHL10,* were suppressed in the pH-buffered medium but not in the non-buffered conditions of Col-0 and an *efr-1* mutant lacking immunity receptor EFR in recognizing EF-Tu, a potent pathogen- or microbe-associated molecular pattern (PAMP or MAMP) of *A. tumefaciens.* Notably, such immune suppression could also occur in *Arabidopsis* seedlings without *Agrobacterium* infection. In conclusion, our studies uncovered new factors in regulating different stages of *Agrobacterium*-mediated transformation and provided new insights into crown gall disease control and improvement of plant transformation.

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Functional Characterization of *Medicago truncatula* Phosphate Transporter 4 in Arbuscular Mycorrhizal Symbiosis

Wei-Yi Lin
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Arbuscular mycorrhizal fungi (AMF) are obligate symbionts which acquire mineral nutrients via their hyphae in exchange for the carbon source from plants. The mutual beneficial relationship has been evolved for more than 400 million years. In root cortical cell, the fungal hyphae form the highly branched structure, called arbuscule, which is encircled by peri-arbuscular membrane (PAM) derived from plant cell membrane. The enlarged plant-microbe interface provide the platform for nutrient exchange. *Medicago truncatula* phosphate transporter 4 (MtPT4), a AM symbiosis (AMS)-specific phosphate (Pi) transporter, is localized in PAM for taking up fungal Pi. Loss-of-function of MtPT4 leads to early degeneration of arbuscules and the reduction of colonization efficiency, suggesting that MtPT4 is essential for arbuscule survival. In addition to transcriptional regulation, our result also showed the possibility of post-translational regulation of MtPT4, implying the importance of tight control of MtPT4. *AMT2;3* gene encodes an AMS-induced ammonium transporter and mutation on this gene did not affect arbuscule maturation. Surprisingly, in low N conditions, only *mtpt4 amt2;3* mutants but not *mtpt4* single mutants showed premature arbusuclar degeneration phenotype. Latest study further revealed the transcriptional regulation of arbsucule degeneration controlled by different combination of transcription factors. In conclusion, symbiotic signal may be triggered by fungal P and/or N delivering via MtPT4 and MtAMT2;3 which directly or indirectly affects the activity of downstream transcription factors to determine the life of arbuscule.

Reference

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Modulators of the *Arabidopsis thaliana* Innate Immunity Response

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Diseases caused by microbial pathogens significantly contribute to the overall loss in crop yield jeopardizing worldwide food security. To sense invaders, plants are equipped with surveillance machineries such as plasma membrane surface-localized proteins called pattern recognition receptors (PRRs), which detect microbe-associated molecular patterns. In order to better understand plant resistance to pathogens, my laboratory analyses the function of key immunity proteins that are part of PRR complexes and are involved in the *Arabidopsis thaliana* defense response. Notably, we study the role of Lectin Receptor Kinases, Leucine-Rich Repeat Receptor Kinases and Cysteine-Rich Receptor-Like Kinases in plant innate immunity. I will present selected data stressing the key role of these receptor kinases in plant defense and their possible use as tool to increase crop resistance to deleterious microbes.

BAK1, a key regulator in plant development and immunity

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Brassinosteroids (BR) are essential phytohormones regulating multiple aspects of plant growth, development, and stress adaptations. Within the last two decades, major regulatory components mediating BR biosynthesis and signal transduction have been identified. Mutants deficient in BR biosynthesis or signal transduction show characteristic phenotypes including extremely dwarfed statues, male sterility, delayed flowering, round and epinastic leaves, and altered photomorphogenesos and skotomorphogenesis. We performed a large scale activation tagging genetic screen trying to identify extragenic suppressors of a weak BR receptor mutant, *bri1-5*. *bak1-1D* is one of the suppressors we identified (Li et al., 2002). With the last 16 years, my lab has been focusing our research on the functional analyses of BAK1 in regulating BR signal transduction as well as other signaling pathways. For example, using genetic and biochemical approaches, we found that BAK1, acting as a co-receptor of the BR signaling, plays an essential role in the BR signal transduction (Gou et al., 2012). We also found that BAK1 regulates multiple pathways in development and immunity (He et al., 2007; Ou et al., 2016). I will present some of the recent progress we are making in understanding the detailed molecular mechanisms of BAK1 in regulating these distinctive signaling pathways.

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Try to remember: Regulation of Heat Stress Memory in Plants

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Heat stress (HS) episodes, varied in magnitude and duration in the natural environment, are detrimental to plant development and growth. Plants are sessile and need to cope with various HS conditions by triggering counteracting thermotolerance mechanisms, including HS memory. HS memory was first observed in studying the Arabidopsis loss-of-function mutant of HSA32, which is a heat-inducible protein conserved in land plants. In Arabidopsis seedlings, acquired thermotolerance (AT) conferred by heat acclimation lasts for a short period and gradually decays within 2-4 days to pre-acclimation level. This phenomenon allowed us to develop the long-term AT (LAT) assay, which revealed that AT decays much faster in HSA32 knockout mutant than in the wild type (Charng et al., 2006). Since then, reverse and forward genetic studies using the LAT assay have facilitated the identification of other components associated with HS memory in Arabidopsis. A diverse set of molecules has been shown to involve in extending the memory of heat acclimation, including transcription factors (Charng et al., 2007; Lämke et al., 2016), protein chaperones (Lin et al., 2014; Sedaghatmehr et al., 2016; Wu et al., 2013), and micro-RNA (Stief et al., 2014). In our lab, we study two regulatory pathways of HS memory. The first one involves an HSFA1 and HSFA2 transcription cascade, which regulates the transcription memory of many HS response genes (Liu et al., 2018). The second one involves a positive feedback loop at a post-transcriptional level between HSP101 and HSA32. In this talk, recent progress in studying these two pathways will be presented and discussed.

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Effect of LiCl on salt stress response in *Arabidopsis thaliana*

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Plants respond to abiotic stresses through specific signal transduction pathways. Under condition of salt stress, abscisic acid (ABA)-related gene expression up-regulates and modulates stress response in plants. Ethylene is also known to be involved in salt stress response. In our previous study, we found that Arabidopsis ACC synthase *ACS7* is involved in root gravitropism. Compared to wild type, *acs7* mutant line was less sensitive to the inhibition of root gravitropism by a calcium channel blocker LiCl, a cure known to bipolar disorder in humans. However, how plants respond to LiCl is little known. In an independent study, *acs7* mutant line was reported to be salt tolerant. After salt stress treatment, survival rate and seed germination rate of Arabidopsis was reduced. With LiCl treatment under salt stress condition, however, reduced seed germination rate was recovered. In comparison to wild type, gene expression of ABA-related marker genes *i.e. KIN1, KIN2* exhibited different level in *acs7* mutant. These suggest that LiCl may affect cross talk between ABA and ethylene signaling pathways in plants.

Role of the Rice Transcription Factors OsbHLH068 and OsbHLH035 in Flowering and Salinity Stress

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Many transcription factors (TFs), such as the basic helix-loop-helix (bHLH) gene family, are important for regulating plant growth and responses to abiotic stresses. However, most of *OsbHLHs* gene functions are still unknown in rice. We are interesting to understand the functions of two rice transcription factors, OsbHLH068 and OsbHLH035, in flowering and salt stress. These two gene expressions were induced under salinity stresses. Heterologous overexpression of *OsbHLH068* in *Arabidopsis* delayed seed germination, reduced salt-induced H_2O_2 accumulation, and promoted root elongation, which is contrary to the knock-out mutant phenotype of its homologous gene *AtbHLH112*. However, transgenic *Arabidopsis* seedlings overexpressing *OsbHLH068* and *Atbhlh112* mutant all showed the same late-flowering phenotype. Further analysis by microarray and qPCR revealed that the expression of *FT* gene was down-regulated in both *OsbHLH068*-overexpressing *Arabidopsis* plants and *Atbhlh112* mutant plants, whereas *SOC1* but not *FT* was highly expressed in *AtbHLH112*-overexpressing *Arabidopsis* plants. Several stress-responsive genes, such as *AtERF15* and *AtPUB23*, were also affected in *OsbHLH068*- and *AtbHLH112*-overexpressing transgenic *Arabidopsis* plants. Besides, we found that *Osbhlh035* Tos17 mutant showed a delay seed germination phenotype under salt stress. The abscisic acid (ABA) content was over-accumulated, and the expression of *OsABA2* and *OsAAO3* was induced, while *OsABA8ox1* in germinating *Osbhlh035* mutant seeds was repressed. Furthermore, *Osbhlh035* mutant seedlings could not recover from salt-stress because sodium accumulated excessively in shoots of *Osbhlh035* seedlings after salt treatment. Interestingly, the gene expression of the sodium transporters *OsHKT1;3* and *1;5* was reduced in *Osbhlh035* shoots and roots, respectively. In conclusion, our studies indicate that *OsbHLH068* and *AtbHLH112* have partial redundancy in salt stress response, but have opposite functions in controlling *Arabidopsis* flowering. Meanwhile, *OsbHLH035* mediates ABA-dependent seed germination and ABA-independent seedling recovery from salt stress by activating *OsHKT* genes expression.

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