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Abstract Booklet

Functional study of the zinc finger protein TZF11 in brassinosteroid-regulated plant growth in *Arabidopsis*

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Brassinosteroids (BRs) are important steroidal hormones that regulate numerous developmental processes in plants, including cell expansion, cell division, senescence, male fertility, fruit ripening, and responses to environmental stresses. BR signaling is mediated by a signaling pathway composed of the BR receptors BRI1-BAK1, transcription factors BZR1-BES1, and several other signaling components. As a key transcription factor and positive regulator of BR signaling, BZR1 has been shown to mediate BR crosstalk with many other signaling pathways in plants. From a BZR1-based yeast two-hybrid screen, we identified a BZR1-interacting protein TZF11 which is a CCCH-type zinc finger protein with putative transcriptional regulatory activity. TZF11 and family proteins have been previously reported to be involved in plant response to abiotic stresses such as salinity; however, their functions in plant growth have not been addressed. Our recent data show that TZF11 interacts with BZR1 in vitro and in vivo and overexpression of TZF11 inhibits plant growth. TZF11 accumulation in plants is BR-inducible and overexpression of TZF11 suppresses BZR1's activity in regulating plant growth, target gene expression and responses to BR treatments. Our results suggest that TZF11 may represent a new negative regulator of pant growth and BR signaling.

Molecular Mechanisms of Plant Autophagy and Autophagosome Formation

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Autophagy is an essential metabolic process which mediates the degradation of the damaged organelles and unwanted cellular contents. During autophagy, a double membrane bound organelle called autophagosome will form and deliver the cargos into vacuole or lysosome for degradation. In plants, autophagic process relies on autophagy-related (ATG) proteins to maintain the basal level of nutrient recycling, whereas depletion of ATG genes causes a direct impairment of plant yield, flowering time and seed quantity/quality. Therefore, understanding how various ATG proteins orchestrate is a central point in autophagy study.

Our lab had previously demonstrated that *At*ATG9 functions in ER (endoplasmic reticulum)-derived autophagosome formation and regulates *At*ATG18a in a PI3P-dependent manner in *Arabidopsis*. Besides ATG proteins, *Arabidopsis* SH3 DOMAIN-CONTAINING PROTEIN 2 (SH3P2) is a PI3P-binding protein which interacts with ATG8 and involves in autophagosome formation at the ER. According to the yeast model, the ATG9-ATG18-ATG2 complex plays pivotal role in preautophagosomal structure (PAS) targeting to promote autophagy. The *Arabidopsis* genome contains one *At*ATG9, one *At*ATG2 and eight *At*ATG18 (a/b/c/d/e/f/g/h), however, their underlying mechanisms in regulating autophagosome formation in plants remain elusive.

Toward this goal, we used yeast two hybrid screening to identify the specificity of *At*ATG18a and its relationship with *At*ATG2. In addition, results from both cellular and biochemical experiments suggest that autophagic flux is severely hampered in *atg18a* and *atg2* mutants. The subcellular localization ratio of YFP-*At*ATG18a and RFP-*At*ATG8e is dramatically increased under *atg2* compared with that in the wild type (WT) plant, indicating the possible role of *At*ATG2 in mediating *At*ATG18a dynamics. Structural transmission electron microscopy (TEM) analysis will be performed in these mutants to further illustrate their possible effects on autophagosome formation. Interestingly, the degradation of peroxisome is also affected in *atg18a* and *atg2* mutants, suggesting that both *At*ATG18a and *At*ATG2 may play roles in the selective removal of damaged peroxisome, a process termed pexophagy. Taken together, results obtained so far suggest that *At*ATG2 works together with *At*ATG18a, and regulates the dynamics of *At*ATG18a in autophagosome biogenesis and peroxisome degradation in plants.

The impact of higher energy output from chloroplasts or mitochondria on Arabidopsis physiology

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Arabidopsis thaliana purple acid phosphatase 2 (AtPAP2) is a protein dully anchored on the outer membranes of chloroplasts and mitochondria. Overexpressing of *AtPAP2* simultaneously modulates the physiology of mitochondria and chloroplasts, and results in faster growth and higher seed yield. Here we generated transgenic lines that overexpressed *AtPAP2* solely in mitochondria (P2TOM lines) or chloroplasts (P2TOC lines), respectively.

Compared to the WT, P2TOM lines exhibited early senescence, lower seed yield, lower sucrose but higher ATP contents under illumination. While overexpression of *AtPAP2* on mitochondria increased its activity via modulating the TCA pathway, the P2TOM lines exhibited a lower value of ETR and enzyme activity of the key enzymes in the Calvin cycle, inferring a lower LEF and carbon fixation. Thus, a higher mitochondrial activity with limited carbon supply from the chloroplasts could explain the phenotypes of the P2TOM lines. By contrast, the P2TOC lines exhibited higher ETR and abundances of some key enzymes in the Calvin cycle. Interestingly, the abundances of some TCA enzymes were also increased but with a lower abundance compared to the OE line. Meanwhile, the ATP content in P2TOC decreased at the first beginning of illumination, then increased with the prolonged illumination time at middle of the day compared to WT, which implies the potential ability of activated chloroplasts could strengthen the function of mitochondria for ATP generation. This could explain why the growth rate and seed yield of the P2TOC line are higher than the WT but lower than the OE line.

In conclusion, the energy status in plant cells are balanced by the activities of both chloroplasts and mitochondria as they are the producers and consumers of carbons and reducing equivalents respectively.

Understanding homoeologous proteins interactions for defense signaling in *Arabidopsis* allotetraploids

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The goal of my research is to understand the contribution of homoeologous proteins interactions for improving defense responses in hybrids. We have recently published a paper showing improved defense response in a resynthesized *Arabidopsis* allotetraploids (*Arabidopsis suecica*) when compared to its autotetraploid progenitors, *Arabidosis thaliana* (At4) and *Arabidopsis arenosa* (Aa). We found that differential expression and preferential alleles expression of *WRKY18* and *WRKY40* in the allotetraploids is correlated with the observed resistance against the bacterial pathogen, *Pseudomonas syringae,* in the allotetraploids. Furthermore, we have demonstrated that preferential *cis*-interacting homodimeric and *trans*-interacting heterodimeric interactions were capable of inducing differential expression of the WRKYs' targets and *PR1* expression in *Arabidopsis*. Here, we hypothesize that such *cis-/trans*-interaction between the WRKY18 and WRKY40 alleles from At4 and Aa could alter the downstream signaling cascade involving defense response in the allotetraploids. To test this, we propose to identify the targets and gene expression changes associated with various WRKY18 and WRKY40 homo-/ hetero-dimer interaction (in *cis* or *trans*) at a genome-wide level. An estrogen-inducible system will be used to control the ectopic overexpression of epitope-tagged (HA or Myc) At/Aa WRKY18 or WRKY40 alleles in transgenic *Arabidopsis*. ChIP-seq and mRNA-seq will then be used to identify the direct targets and defense signaling networks mediated by various type of *cis*- or *trans*- interacting WRKY homo-/hetero- dimers *in vivo*. It is expected that results obtained will provide a more comprehensive insight to the contribution of homoeologous alleles interaction and defense response in plant hybrids.

Development and verification of a biophysical crop model with explicit implementation of ozone damage

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It is projected that food production must increase by at least 50% by year 2050 to meet the growing global food demand. However, food security is threatened by both global warming and worsening ozone air pollution, which are shown to harm photosynthesis and crop growth. A common approach to estimate ozone-induced yield losses is to use empirical ozone exposure metrics and crop-ozone concentration response functions. Such approach typically does not account for crop ecophysiological responses to ozone uptake along with other covarying factors such as rising CO_2, heat waves and droughts, which may all induce stomatal closure that reduces ozone uptake. Here we examine ozone-induced yield loss and its covariations with other ecophysiological factors by implementing a photosynthesis-based stomatal ozone uptake and ozone damage algorithm in the Terrestrial Ecosystem Model in R (TEMIR). TEMIR is an ecophysiological model developed in-house and driven by prescribed surface micrometeorology. We first validate and optimize TEMIR with site-level gross primary production (GPP), leaf area index (LAI), crop yield data and canopy-level stomatal ozone uptake flux by implementing and testing different combinations of phenology and carbon allocation schemes derived from two global biophysical crop models, CLM4.5-crop and JULES-crop. We find that implementing increased leaf senescence rate, delayed leaf emergence and ozone damage on photosynthesis substantially improves agreement with observed crop variables in Nebraska, U.S., for 2001–2003 over the default algorithms. Driven by hourly ozone data from US EPA, stomatal ozone uptake during the same period is simulated to reduce maize yield by 15.1 – 25.5% and soybean yield by 21.9% which is overestimated compared to the yield loss compute from AOT40 metric. Growing season GPP for maize and soybean is reduced by 26.1 – 40.6% and 22.7%, respectively. In this regard, a biophysical model with ecophysiological representations of ozone-CO_2-crop interactions is necessary to evaluate how crop production and food security will evolve in a changing climate.

Special genomic and hormonal features in millipedes

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The biology of non-insect and non-crustacean arthropods has been quite ignored and understudied for a relatively long period time. Millipedes being one of the arthropods, are a monophyletic group that are characterised by possessing two pairs of jointed appendages on most of the body segments, and are recognized scientifically as the class Diplopoda. Despite the advent of research in insects and crustaceans models, that in myriapods, especially millipedes, remains rather enigmatic. Here, we show two findings in this class of organism using two representative species from distinct orders, *Helicorthomorpha holstii* and *Trigoniulus corallinus*.

First, two argonaute protein (AGO) coding genes were identified in both the millipedes and their gene sequences show high similarity with those in other arthropods. Yet in addition, we are able to identify and prove that the AGO2 gene in *T. corallinus* has incorporated multiple stop codons in the PAZ (nucleic acid binding) domain, possibly causing a truncation in gene expression. We believe that AGO2 has become a pseudogene and this is the first case being discovered and proved in invertebrates. Second, we show that the ortholog of juvenile hormone acid O-methyltransferase (JHAMT), an essential biosynthesis enzyme methylating farnesoic acid (FA) to methyl farnesoate (MF), and catalyzing MF to juvenile hormone (JH), could not be found in multiple millipede genomes. We hypothesized that millipede thus might be utilizing FA as the growth regulator rather than MF and JH being identified previously in other arthropods.

Our findings, thus provide, to our knowledge, first unexpected genomic and hormonal features in this previously ignored group of arthropods, and suggest that this class is unique and deserve further investigations in facilitating the understanding of the evolution and development of arthropods.

Functional study of *Yin Yang 1* in mouse central nervous system development

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The highly conserved and ubiquitously expressed transcription factor Yin Yang 1 (YY1), was named after its dual roles of both activating and repressing gene transcription. *Yy1* plays complex roles in various fundamental biological processes such as cell cycle, cell proliferation, survival, and differentiation. Mouse embryos encounter peri-implantation lethality when *Yy1* was completely inactivated. Ablation of *Yy1* in *Xenopus* embryos indicates it is essential for neural induction and patterning. Previous studies have revealed that *Yy1* is critical for stem cell development in different tissues as well. However, studies focus on the role of *Yy1* in central nervous system (CNS) development remain limited.

As a model system to study the CNS, the cerebellum consists of defined types of cells, and has the well-known functions of motor control, cognition, and emotion. The isthmus organizer locates at the mid-hindbrain boundary (MHB) region serves as the critical signaling center during cerebellar early patterning. The Purkinje cell (PC) is the sole output neuron of the mature cerebellum. They undergo dendritic remodeling within the early postnatal weeks. The proper maturation of dendritic tree is extremely important for the function of the cerebellar neural circuit. Disruption of the cerebellar developmental processes may cause severe CNS disorders.

Yy1 expresses universally in the developing mouse cerebellum. Using tissue-specific *Cre-LoxP* system, we generated conditional knockout mouse lines to inactivate *Yy1* in the MHB and in the PCs, for studying the function of *Yy1* in neural stem cell (NSC) specification and neuronal maturation respectively. Mice with *Yy1* deletion in the MHB region displayed cerebellar agenesis and loss of the posterior midbrain. The *Yy1* deleted cells underwent cell cycle arrest and apoptosis, with the concurrent change of cell cycle regulatory genes expression, as well as the accumulation of Trp53. Mutant mice with PCs-specific *Yy1* knockout showed severe motor defect due to improper formation of PC dendrites, but no defect in survival of PCs. These results suggest the differential regulatory roles of *Yy1* in NSCs and neurons. We shall continue to investigate these mechanisms in the future.

TRPC3 contributes to the proliferation and apoptosis resistance of triple negative breast cancer cells

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Currently there is no effective molecular-based therapy for triple-negative breast cancer (TNBC) which is a kind of highly metastatic and aggressive breast cancer subtype. Differential expression as well as dysregulation of specific Transient receptor potential (TRP) channels have presented positive correlations with different breast cancer subtypes. Previous studies showed that up-regulated TRP channels worsen breast cancer progression through increasing cell proliferation, migration and invasion. TRP channels have been proposed as potential breast cancer diagnostic markers and therapeutic targets.

A Ca^{2+}-permeable channel canonical TRP channel-3 (TRPC3) was reported to contribute to ovarian cancer progression. TRPC3 was reported to be up-regulated in breast cancer biopsy tissues when compared to normal breast tissues. While the biological role of TRPC3 in breast cancer has never been elucidated.

In our current study, MDA-MB-231, which is estrogen receptor (ER) –, progesterone receptor (PR) – and human epidermal growth factor receptor 2 (HER2) –, and MCF-7, which is ER+, PR+/–, HER2–, were chosen as cell models for *in vitro* research. Western blot and immunocytochemistry showed that TRPC3 was over-expressed on the plasma membrane of TNBC cell line MDA-MB-231 when compared to estrogen receptor positive cell line MCF-7. TRPC3 blocker Pyr3 and dominant negative (DN) of TRPC3 in MDA-MB-231 attenuated cell proliferation, induced cell apoptosis and sensitized cell death to chemotherapeutic agents as measured by proliferation assays. Flow cytometry confirmed that TRPC3 blocker Pyr3 increased DNA damage with accumulation of sub-G1 phase but did not affect cell cycle distribution of viable cells. Blocking TRPC3 induced cell apoptosis in a caspase-dependent manner as indicated by the cleavage of caspase-7 and PARP proteins. Importantly, phosphorylated p38 MAPK, ERK1/2 and JNK proteins in the mitogen-activated protein kinase (MAPK) pathway were all up-regulated due to TRPC3 blockade. These results suggest that TRPC3 contributes to the cell proliferation of MDA-MB-231 cells and acts as an anti-apoptotic regulator in the protection of cell death.

This study reveals a new target (TRPC3) for advanced breast cancer therapy.

The Role of Type 2 Innate Signals in Tissue Regeneration

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Mammalian adult cardiomyocytes lose their proliferative capabilities during post-neonatal development, and is the primary factor to failure in regenerating tissue following an adult myocardial infarction. Recent findings using neonatal ischemic heart models have revealed the indispensability of macrophages to resolve injury in the heart, while studies characterizing neonatal immunity describes an immature, intrinsically Th2-skewed immune profile. However, the interplay between neonatal preference for a type 2 immune response and an active cardiomyocyte population on heart regeneration is not yet fully understood. Thus, we aim to understand the effects of major drivers of Th2 immunity on the neonatal heart following injury, specifically the type 2 signals: interleukin-4 (IL-4) and interleukin-13 (IL-13).

We utilized an IL-$4^{-/-}$/IL-$13^{-/-}$ knockout (DKO) mouse to observe how loss of IL-4 and IL-13 would impact regeneration after neonatal cardiac injury. To mimic an ischemic event, the neonatal left anterior descending coronary artery was ligated on the second day after birth. Subsequently, echocardiography, flow cytometry and transcriptional analysis were performed on both WT control and DKO neonatal hearts at various time points after injury.

Following cardiac injury, we observed a significant decline in cardiac function via ejection fraction and fractional shortening in the DKO compared to WT neonates, which fully recovered. Flow cytometry analysis indicated that DKO neonates increased $CD8^{+}$ cytotoxic T-cells, recruited greater numbers of pro-inflammatory $Ly6C^{HI}$ monocytes and reduced the number of anti-inflammatory macrophage populations compared to WT controls. Transcriptional analysis comparing WT and DKO injured neonatal heart tissue indicated greater expression of pro-inflammatory cytokines such as TNFα and lower expression of anti-inflammatory markers such as Fizz1 in DKO neonates. Moreover, an examination of HuR expression, a protein that stabilizes pro-inflammatory cytokine mRNA, indicated a significant increase in expression, while pro-angiogenic markers such as VEGF expression and the number of endothelial cells were significantly reduced in DKO neonates.

These results all indicate an inability of DKO mice to maintain a Th2 preferential response to ischemic injury observed in WT controls; suggesting that IL-4 and IL-13 inhibit pro-inflammatory responses predicated on the stabilization of pro-inflammatory cytokines which attenuate regenerative programs such as angiogenesis.

Comprehensive Identification of Soybean Long Noncoding RNAs and Functional Implications in Stress Responses

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Long non-coding RNAs (lncRNAs) are generally defined as non-protein coding transcripts that are at least 200 nucleotides in lengths. They are known to play pivotal roles in regulation of gene expression at all levels. In plants, some studies have also implicated their possible roles in stress responses. We took advantage of a large collection of in-house transcriptome data on different soybean tissues under various treatment conditions to perform a comprehensive identification of soybean lncRNAs. We also retrieved all existing soybean transcriptome data from public sources that are of sufficient quality and sequencing depth to enrich our analysis. In total we have 241 datasets for this analysis. An integrated reference-based and *de novo* transcript assembly was developed, which identified 1,521 core lncRNA gene loci encoding 1,540 transcripts. Differential gene expression and co-expression analyses revealed that lncRNA genes may participate in phosphate transportation and auxin related transcription regulation upon salt and drought conditions. By incorporating DNA methylation data, we showed that down-regulation of lncRNA genes upon salt stress could be linked to DNA hypermethylation of their upstream regions, while these DNA hypermethylation events were postulated to be led by up-regulation of RNA dependent DNA methylation related RNA polymerase subunits or down-regulation of DNA demethylase.

Current definition of “non-protein coding” in a transcript refers to the absence of open-reading frame (ORF) of at least 100 amino acid residues in length. However, small ORFs that encode around 10 amino acids were routinely predicted in the lncRNA sequences. We performed small peptides extraction from soybean root tissues, and preliminary mass spectra data suggested the translation and phosphorylation of two small peptides encoded by two lncRNA genes. This observation indicated that some of the so-called noncoding genes in the transcriptome could be in fact coding for small peptides, which provides a new layer of functions to this class of enigmatic transcripts inside the cells.

Investigation of Epigenetic Features in Gastric Cancer

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Epigenetics has been defined as the "molecular factors and processes around DNA that are mitotically stable and regulate genome activity independent of DNA sequence" (Finnegan et al. 2000). Epigenetic research examines biochemical modifications of the genome and aims to determine the resulting consequences on gene function. The two most commonly studied epigenetic mechanisms are DNA methylation and histone modifications.

Gastric cancer is the third leading cause of cancer-related death worldwide (Rugge et al. 2015), and in Asia, Gastric Cancer is the third most common cancer after breast and lung and is the second most common cause of cancer death after lung cancer (Rahman et al. 2014). Currently, our knowledge of epigenetic alterations of Gastric Cancer remains limited. Here, we will depict and compare the epigenetic alterations in Gastric Cancer samples and normal samples, including five types of histone modifications and DNA methylation. This research is focusing on the epigenetically dysregulated genes which were significantly involved in the hallmarks of Gastric Cancers, and the pattern of epigenetics modifications in tumors samples. The dataset of this research will be integrated with RNA-seq data (gene expression), ChIP-seq data (histone modifications) and HumanMethylation450 data (DNA methylation). The purpose of this research is to discover the epigenetically dysregulated hallmark genes and to reveal some regular patterns of histone modifications and DNA methylation in Gastric Cancer.

The R packages (Bioconductor) provide us the methods to test for differential histone modifications and differential methylation (Huber et al. 2015). Meanwhile, the distributions of histone modifications and DNA methylation in the regions flanking transcription start sites (TSSs) for genes of the whole genomes as well as oncogenes are computed, respectively. This study will provide valuable resource for investigations at understanding epigenetic regulation of Gastric Cancer.

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A high-quality Reference Genome of Wild Soybean

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Deciphering genetic blueprint of wild soybean is important for cultivated soybean germplasm improvement since wild soybean preserves abundant genetic diversity that has been lost during domestication. Though efforts have been made to construct genome assembly for wild soybean, the publicly available assemblies were highly fragmented and hence hindered wild soybean related studies.

In my study, a high-quality reference genome of the wild soybean W05 was constructed using a combination of methods including single-molecule real time sequencing (PacBio), next-generation sequencing (Illumina), optical mapping (Bionano), and Hi-C sequencing (Dovetail). The final assembly of W05 is 1,013.2 Mb in length, with contig N50 of 3.3 Mb. In total, 89,755 protein-coding transcripts were annotated for 55,567 gene loci in W05 genome. Comparative genomic analysis reveals massive genetic variations between wild soybean W05 and cultivated soybean genomes. Comparing with wild soybean W05, 32 and 12 large structural variations (>100Kb) were identified in cultivated soybeans Wm82 and ZH13, including previously reported reciprocal translocation between Chr11 and Chr13. In addition, an inversion was identified at the seed coat color controlling *I* locus in both Wm82 and ZH13, which is responsible for soybean seed coat color transition during domestication. Moreover, genome wide accession-specific transposon elements insertion was identified. Results shows that genes affected by TEs insertion are enriched in metabolic biological process for cultivated soybean Williams 82 and ZH13.

In summary, a high-quality reference genome was assembled for wild soybean W05. It will be invaluable resources for wild soybean related studies and cultivated soybean germplasm improvement.

Investigation of Soybean Histone Code by Top-down Proteomics Approach

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Histone code refers to amino acid post-translation modifications (PTMs), both singularly or in combination, in histone. They are important marks and regulators of gene expressions and chromatin folding. Apart from changing the openness of DNA around the histone for transcription factors to access, they could also act as binding motif for regulatory proteins. Hence, histone code plays an crucial role in shaping the identity and the growth and survival of any cell. Histone codes are utterly complex, in which various modifications on multiple residues could work collectively to bring about different effect. Up to this moment, histone code remains as a new field, in which tremendous information about the modifications' combinations, functions, and mechanisms are still waiting to be defined.

Soybean is an essential crop worldwide that are proven to be beneficial to both human and the nature. Yet, the cultivated strain of soybean is sensible to saline soil, making yields in part of the world compromised. Recently, it has been discovered that the histone code in soybean plants plays a great role in conferring salt resistance to the plant.

Previously, our group have identified the changes in some of the soybean plant's histone PTMs under salt stress. We have identified some interacting proteins of these PTMs and proposed a model about their mechanism and effects. Recently, we are developing a high-throughput and high-resolution top-down proteomic platform to analyse the full PTM pattern of Histones, by using nLC-Orbitrap. In the coming future, we will utilize this platform to re-visit our previous findings, as well as to resolve the dynamics of the complete histone PTM patterns under salt stress. At the end of the day, we aim to decipher the histone code that is responsible to confer salt resistance to soybean plants.

The C-terminal of Arabidopsis RMR1 is an E3 ligase

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The plant receptor-homology-transmembrane-RING-H2 (RMR) proteins are type I transmembrane proteins, which are involved in sorting soluble cargo proteins to the protein storage vacuole (PSV). The C-terminal region of RMR (RMR-CT) is involved in the trafficking of the receptor to the PSV, but the biological function of the RMR-CT proteins and how it directs RMR to PSV in plant cells is poorly understood.

We firstly resolved the crystal structure of RING domain of AtRMR1 (AtRMR1-RING). Sequence analysis combined with the structural comparison showed that the AtRMR1-RING domain shares significant sequence-structure homology to the RING domain of known E3 ubiquitin ligases. The in vitro ubiquitination assay system showed that AtRMR1-RING has an E3 ubiquitin ligase activity. Mutation of conserved residues I234 in AtRMR1-RING that may involve in the binding of E2 ubiquitin-conjugating enzymes indicated that I234Y mutation could break the ligase activity in vitro.

More interestingly, our data further showed the self-ubiquitination of C-terminal region of AtRMR1 (AtRMR1-CT), which means that AtRMR1-CT contains the substrate of ubiquitin. The created AtRMR1-CT K288A mutation abolished the self-ubiquitination of AtRMR1-CT, proving that Ser-Rich domain of AtRMR1-CT was the substrate of AtRMR1-RING E3 ubiquitin ligase, and K288 residue is the ubiquitin linkage site. And also, our preliminary in vivo results showed that the K288A substitution in AtRMR1-CT increased its co-localization with the Golgi marker (Man1-mRFP) but decreased co-localization with the TGN marker (mRFP-SYP61), it means that poly-ubiquitination of AtRMR1 affects its trafficking. Our findings will provide better understanding how ubiquitination affects protein sorting to the protein storage vacuoles in plant cells. Such knowledge may have biotechnology applications in the future on targeting recombinant proteins to the protein storage vacuoles in crops.

Characterization of an *Arabidopsis* splicing factor and its five transcript isoforms

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Eukaryotic genes contain introns, which need to be removed after transcription to generate mature mRNA for protein translation. One pre-mRNA can generate multiple mRNA transcripts by alternative splicing (AS). As a result, protein isoforms encoded by the mRNA variants might be structurally and functionally different, due to the presence or absence of certain motifs. AS is a common type of post-transcriptional regulation in eukaryotes and contributes to transcriptome diversity. However, among the large amount of the alternative isoforms detected so far, only a small portion have been studied. It remains unclear that how many transcript isoforms are translated and have biological functions in protein level.

Serine/Arginine-rich (SR) proteins are a major family of splicing regulators required in AS. Interestingly, SR genes themselves are often transcribed into multiple transcript isoforms, which may add to the complexity of AS regulation. *SR184* is an *Arabidopsis* SR gene that has at least seven transcript isoforms encoding five hypothetical proteins (named as isoform 1-5). The full length SR184 is a multidomain protein and differed domain organizations can be observed among its five isoforms. In this research, we aimed to explore the biological roles of these SR184 isoforms. Our preliminary results showed that the *SR184* gene was mainly expressed in *Arabidopsis* seed and silique, with slight differences among five transcript isoforms. Upon heat treatment, most *SR184* mRNA isoforms showed different degrees of increase while *SR184.2* remained unaffected. When transiently expressed in *Arabidopsis* protoplast, SR184.1, SR184.2 and SR184.3 showed nucleus localization while SR184.4 was distributed in punctate signals in cytoplasm. Different properties displayed by these *SR184* isoforms at either mRNA or protein level suggested that they might possess different functions.

The function of HMGS in plant development and isoprenoid production

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Isoprenoids are a large group of natural products with diverse functions. For example, cytokinins (CKs), sterols, brassinosteroids (BRs), precursors of abscisic acid and dolichols are known to regulate plant growth and development. Dietary phytosterols had been reported to lower blood cholesterol levels. Carotenoids and vitamin E are health-promoting compounds because of their high antioxidant activities. In plants, isoprenoids are biosynthesised from the cytosolic mevalonate (MVA) pathway and the plastidial 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway. 3-Hydroxy-3-methylglutaryl-CoA synthase (HMGS) is the second enzyme in the MVA pathway. Previous studies demonstrated that recombinant *Brassica juncea* HMGS (S359A) displayed a 10-fold higher enzyme activity. The overexpression of wild-type (wt) and mutant BjHMGS1 (S359A) (OE-wtBjHMGS1 and OE-S359A) in Arabidopsis up-regulated several genes in sterol biosynthesis and increased sterol content. Furthermore, tobacco OE-wtBjHMGS1 and OE-S359A showed enhanced sterol content, plant growth and seed yield. Herein, transgenic tomato overexpressing wt and mutant BjHMGS1 (S359A) showed increased plant growth, corresponding to a higher expression of BR-, CK- and dolichol-related genes. Also, tomato OE-wtBjHMGS1 and OE-S359A fruits accumulated more MVA-derived squalene, phytosterols, and MEP-derived carotenoids and α-tocopherol (vitamin E), demonstrating crosstalk between the MVA and MEP pathways. This study provides a promising strategy to concurrently increase health-promoting squalene, phytosterols, carotenoids and α-tocopherol in crops.

Thylakoid-Bound Polysomes and a Dynamin-Related Protein, FZL, Mediate Critical Stages of the Linear Chloroplast Biogenesis Program in Greening Arabidopsis Cotyledons

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Biogenesis of the complex 3D architecture of plant thylakoids remains an unsolved problem. Here, we analyzed this process in chloroplasts of germinating Arabidopsis thaliana cotyledons using 3D electron microscopy and gene expression analyses of chloroplast proteins. Our study identified a linear developmental sequence with five assembly stages: tubulo-vesicular prothylakoids (24 h after imbibition [HAI]), sheet-like pregranal thylakoids that develop from the prothylakoids (36 HAI), pro- liferation of pro-grana stacks with wide tubular connections to the originating pregrana thylakoids (60 HAI), structural differ- entiation of pro-grana stacks and expanded stroma thylakoids (84 HAI), and conversion of the pro-grana stacks into mature grana stacks (120 HAI). Development of the planar pregranal thylakoids and the pro-grana membrane stacks coincides with the appearance of thylakoid-bound polysomes and photosystem II complex subunits at 36 HAI. ATP synthase, cytochrome b6f, and light-harvesting complex II proteins are detected at 60 HAI, while PSI proteins and the curvature-inducing CURT1A protein appear at 84 HAI. If stromal ribosome biogenesis is delayed, prothylakoids accumulate until stromal ribosomes are produced, and grana-forming thylakoids develop after polysomes bind to the thylakoid membranes. In fzo-like (fzl) mutants, in which thylakoid organization is perturbed, pro-grana stacks in cotyledons form discrete, spiral membrane compartments instead of organelle-wide membrane networks, suggesting that FZL is involved in fusing membrane compartments together. Our data demonstrate that the assembly of thylakoid protein complexes, CURT1 proteins, and FZL proteins mediate distinct and critical steps in thylakoid biogenesis.

Lifestyle transitions in *Rhizobiales*

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One of the most prominent characteristics of bacteria is their diverse lifestyles. Rhizobiales, a clade of Alpha-proteobacteria with many agriculturally, medically and ecologically important species that have adapted to different lifestyles, provides a good system to study lifestyle evolution. However, to date, most studies are limited to Rhizobiales species of specific lifestyles, and the transition and evolution of lifestyles in the entire Rhizobiales remains largely unexplored. We conducted a comprehensive analysis of ~900 genomes to investigate the evolution and genomic changes of lifestyle transition in Rhizobiales. We classified Rhizobiales lifestyles as nodule association, plant association, animal association, and free living. Reconstruction of ancestral lifestyles reveals ten major origins of host-associated lifestyles, with multiple lineage-specific losses. Using time-calibration, we estimated that the last common ancestor of Rhizobiales originated at 1.5-2.0 Gya (billion years ago), and that the divergence time of the major host-associated clades generally coincided with or postdated the origin of their host. Intriguingly, transitions between lifestyles were characterized by significant changes in genome content: expansions in rhizobia but reductions in animal-associated species. We further identified the genes that are likely important to each lifestyle, implying the roles of parallel gene gains/losses, horizontal gene transfer, and gene duplication in shaping the evolution of Rhizobiales lifestyles.

Arabidopsis bZIP68 plays a role in sensing oxidative stress to balance stress tolerance with growth

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Perturbation of the cellular redox state by stress conditions is sensed by redox-sensitive proteins so that the cell can physiologically respond to stressors; however, the mechanisms linking sensing to response remain poorly understood in plants.

Here we report that the redox-sensitive transcription factor bZIP68 is localized to the nucleus under normal conditions in Arabidopsis seedlings. Upon oxidative stress treatment, some bZIP68 molecules became cytosolic. We also demonstrate that bZIP68 can be oxidized under H2O2 treatment in vitro and in vivo and this oxidation process is dependent on the redox-sensitive Cys320 residue. Furthermore, the bzip68 mutant seedlings grew slower than wildtype but showed better growth compared under oxidative stress induced by methyl viologen (MV).

ChIP-seq data shows that bZIP68 binds to promoter regions containing the G-box elements which are common among the genes involved in stress tolerance and light responses. The ChIP-seq and RNA-seq results indicate that bZIP68 normally suppresses expression of stress tolerance genes to promote growth, whereas its inactivation enhances stress tolerance but suppresses growth. Taken together, our study indicates that bZIP68 might balance stress tolerance with growth through the extent of its oxidative inactivation according to the environment.