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Abstract Booklet

## ***AtMYB30* is a key regulator in *Arabidopsis* seedlings in response to oxidative stress**

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As sessile organisms, plants are continuously saddled with miscellaneous abiotic stress conditions by the inorganic milieu. To overcome such predicament, plant has evolved various adaptation mechanisms, of which the molecular basis can be divided into downstream effectors and upstream regulators, such as kinases and transcription factors (TFs). Water stress, among others, may be the most important environmental factor that molds plant growth, crop productivity, and even species evolution. During last decades, it has been reported that many of the plant TFs play crucial roles in the perception and transduction of abiotic stress signals, yet more TF genes remain undiscovered, as indicated by the ever-growing omics data. To find novel regulators in water stress response network, I chose 79 candidate genes from 2 *Arabidopsis* transcriptome databases under dehydration stress. For each gene, two individual lines of both mutant and over-expression lines were obtained if available. As TFs are always versatile, the homozygous seeds were used for phenotyping against mimic water stress as well as oxidative stress, hypoxia, copper stress and cadmium stress. Fortunately, some novel phenotypes of promising lines were found, especially for the mutant line of *AtMYB30* that shows hypersensitivity to salt stress, cadmium stress and more importantly, to oxidative stress. *AtMYB30* belongs to the subfamily 1 *R2R3-MYBs*, it was first reported as a key regulator in biotic stress response network, yet evidences rises that it may also participate in some abiotic stress response pathways. While the role of *MYB30* in the response pathway of oxidative stress, the common secondary stress of biotic and abiotic stresses, is largely veiled. Further analyses indicate that *MYB30* may be trans-regulated by some subfamily 4 *R2R3-MYBs*, but this hypothesis remains to be tested. And the upstream regulator and downstream genes of *MYB30* will be disclosed by Y1H and ChIP-sequencing respectively. This study may give us new insights on the roles of *MYB30* in plant abiotic stress regulation network which has long been overlooked, and it may give us new clues to solve abiotic stresses faced by agriculturally, economically and environmentally important plants.

## RNA editing in high energy *Arabidopsis thaliana*

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RNA editing in plants is a post-transcriptional process that modifies cytidines (C) to uridines (U) in organellar RNA transcripts. In angiosperms, pentatricopeptide repeat (PPR) and multiple organellar RNA editing factor (MORF) proteins are essential components of the editosome complex. Overexpression of AtPAP2, a phosphatase located on the outer membranes of two important energy-producing organelles, chloroplasts and mitochondria, leads to higher energy outputs from these organelles resulting in higher ATP and sugar levels and increased seed yield and growth rate in *Arabidopsis thaliana*. Yeast two-hybrid and bimolecular fluorescence complementation assays showed that AtPAP2 can interact with seven out of nine MORF proteins of *Arabidopsis thaliana* except with MORF4 and MORF7. RNA-seq analysis was carried out to compare the organellar transcripts of the AtPAP2 overexpression (OE) line with that of the wild-type at three time points. In total, 34 and 510 editing sites were identified in chloroplast and mitochondrial transcripts, respectively. The degrees of editing of most sites do not differ significantly between OE and WT, except some sites on the transcripts of several cytochrome c maturation (Ccm) genes. Particularly, some sites at a conserved tryptophan-rich region (WWD domain) of CcmF<sub>N2</sub> protein, at the C-terminus of the CcmB protein, and at the transmembrane region of CcmF<sub>C</sub> protein. Western blotting of 2D blue native PAGE showed that the patterns of CcmF<sub>N1</sub> polypeptides were different between the OE and WT lines. We postulate that overexpression of AtPAP2 may influence cytochrome c biogenesis by modulating RNA editing through its interaction with MORF proteins.

## Effects of Future Agricultural Ammonia Emission and Deposition on Air Quality through Vegetation Feedbacks

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The world is facing the challenge of feeding an ever-growing population and simultaneous safeguarding the environment. In many parts of the world but very notably in China, excessive amounts of nitrogen fertilizer are applied to maximize crop production, often inducing unintended environmental consequences, including the substantial release of ammonia ( $\text{NH}_3$ ) into the atmosphere. Atmospheric  $\text{NH}_3$  emission of agricultural origins (fertilizer and livestock wastes) accounts for more than 50% of the total emission globally and over 80% for intensive agricultural areas in Asia (Huang et al, 2012). After chemical reaction and transportation in the atmosphere,  $\text{NH}_3$  deposits back onto the terrestrial ecosystem, modifies soil microbial processes and thus enhances vegetation growth. As parameterizations for plant growth, leaf area index (LAI) and canopy height can potentially impact on serious environmental problems such as ozone ( $\text{O}_3$ ) pollution through biogeochemical and biogeophysical pathways. Here, we adopt an asynchronous coupling framework using the Community Earth System Model (CESM) to investigate how agriculture-induced atmospheric  $\text{NH}_3$  emission and deposition affect air quality via biosphere-atmosphere processes. We conduct two scenarios representing current and future agricultural  $\text{NH}_3$  emission and deposition, and then examine the corresponding vegetation responses. With the perturbed vegetation responses, three simulations evaluating the effect on air quality are driven by the change of 1) LAI only, 2) canopy height only and 3) both LAI and canopy height. We find that obvious enhancements for both LAI (around  $0.4 \text{ m}^2 \text{ m}^{-2}$ ) and canopy height (around 0.2 m) happen in the southeastern part of South America, Sub-Saharan Africa, Europe and southern China. Annual surface  $\text{O}_3$  increases in the middle US and northern China through LAI pathway, and increases in broader areas including the most parts of Russia, western Australia and northeastern Canada via canopy pathway. Our results show that agricultural  $\text{NH}_3$  under future scenario has the potential to worsen air quality via vegetation feedbacks, thereby suggesting the need to formulate optimal strategies for future agricultural practices for the sake of the environment.

## ***In vitro* COPII Reconstitution Reveals Novel Insight of *Arabidopsis* ER Export**

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The molecular mechanisms of COPII vesicle formation and function have been well illustrated via the *in vitro* reconstitution system in both yeast and mammal, yet the existence of COPII vesicles in higher plant remains illusive. Interestingly, plant COPII paralogs also outnumber those in other eukaryotes with their functional diversity under investigation. For instance, the *Arabidopsis thaliana* genome contains five small GTPase Sar1 homologs (AtSar1a/b/c/d/x). Our recent study has demonstrated that a single amino acid difference in AtSar1a is pivotal for its specific cellular localization and the formation of the unique AtSar1a/AtSec23a pair, which contributes to its distinct role on cargo ER export. To further investigate the underlying mechanisms and function of this specific COPII pair in plant, here we first reconstituted plant COPII vesicles *in vitro*. By incubating *Arabidopsis*-derived microsomal-enriched fractions and pre-cleared cytosol with the energy regeneration system, vesicles were reconstituted and purified for morphological study via TEM analysis following negative staining or cryo-fixation. The vesicle production was AtSar1- and energy-dependent, and concentrated vesicles with a diameter of 70-100 nm were identified by immunogold labelling with COPII cargo protein Sec22 antibodies, proving its COPII identity. More strikingly, quantitative proteomics analysis via iTRAQ labelling further revealed the existence of AtSar1a-dependent plant-unique COPII vesicles and their potential cargoes, including families of transporters and channels responsible for plant responses upon abiotic stresses. Using genetic approaches, we confirmed that AtSar1a-specific vesicles were essential for seedling growth especially upon abscisic acid treatment. To conclude, this plant *in vitro* reconstitution system provides strong evidences for the existence of COPII vesicles *in planta*, and reveals novel function of distinct COPII vesicle population in higher eukaryotes especially in response to environmental cues. Supported by grants from the Research Grants Council of Hong Kong.

## Lysine-rich Rice Enhanced Musculoskeletal Growth and Development in Young Rats

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Lysine is the first limiting essential amino acid in rice. To tackle this limitation, we developed transgenic rice grains with enhanced free lysine content (lysine-rich rice). In this study, we performed animal feeding experiments to investigate the effects of lysine-rich rice on the growth and development of musculoskeletal system in weaning rats. Rats were fed with lysine-rich rice (HFL1 and HFL2, with 23% and 18% enhanced lysine, respectively), wild-type rice with various amounts of lysine supplements (+0%, +10%, +20% and +40% lysine) or nutrient-rich commercial rat diet for 70 days (n=8 per group). Previously, we had shown that lysine-rich rice could improve the musculoskeletal growth of rats. Here, we performed serum biochemistry analysis to investigate the underlying mechanism leading to better musculoskeletal growth. Our results showed that rats fed on lysine-rich had higher serum level of essential amino acids, especially lysine, leucine and isoleucine; higher serum level of growth hormone IGF-1 and bone formation marker P1NP, and lower serum level of bone resorption marker CTX-1 and muscle growth inhibitor MSTN. Taken together, rats fed on lysine-rich rice showed higher levels of serum essential amino acids and IGF-1, leading to faster musculoskeletal growth. The results suggested that lysine-rich rice may offer an effective means to improve the growth and development of musculoskeletal system of children, especially in developing countries who depend on rice as main staple food.

## Sox9 controls the secretion of cerebrospinal fluid in the developing CNS

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The choroid plexus (CP) is crucial to the development and homeostasis of the CNS by producing cerebrospinal fluid (CSF) and establishing the blood-CSF barrier. Entry of molecules into CSF is determined by the CP epithelium which is a unique monolayer of actively secretory epithelial cells situated at the interface between brain ventricles and blood vessels. Information regarding the molecular mechanisms that control CSF composition is however largely missing. We identified the transcription factor Sox9 was robustly expressed in the mouse CP epithelium during embryogenesis. Using genetic loss-of-function approach, we show that Sox9 is indispensable for proper CP function. Quantification of CSF content revealed an abrupt increase of protein level upon Sox9 inactivation, suggesting the possibility of compromised CSF barrier and deregulated CP secretion in mutant. We found that Sox9 was required for the expression of genes encoding extracellular matrix (ECM) components in the CP during the establishment of CSF barrier. Deficiency of ECM deposition at the CP basal lamina was observed in mutant. Moreover, expression of the hemidesmosome component integrin  $\alpha 6 \beta 4$  heterodimers at the basal lamina was remarkably reduced in mutant CP. Interestingly, the number of exosomal vesicles were substantially lost in mutant CP epithelium. Forced transcytosis across CP epithelium by induced systemic inflammation failed to boost the production of these extracellular vesicles in the mutant, suggesting that the transcytosis machinery was dysfunctioned upon Sox9 knockout. Our results revealed Sox9 played a central role in controlling CSF composition and establishing the blood-CSF barrier.

## Role of TRPC7 in regulating the functions of embryonic stem cell-derived cardiomyocytes

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Ion channels are vital molecular device to maintain the electrophysiological function and calcium homeostasis of cardiomyocytes. Although classical sodium, potassium and calcium channels are intensively studied, many other channels that may also contribute to the subtle regulation of cardiomyocytes remain largely unexplored. Differ from voltage- and ligand- dependent channels, Canonical Transient Receptor Potential channel (TRPC) is a type of non-selective cation channel activated by G protein coupled receptors. TRPC channel widely expresses in different tissues, playing an important role in the maintenance of normal cellular function. The aim of this project is to study the function of TRPC7, the most elusive member in the TRPC family, in cardiomyocytes. Western blotting showed TRPC7 is expressed in mouse heart, and detection of multiple bands indicated that more than one isoforms may exist in heart. Immunocytochemistry experiments showed the channel locates at plasma membrane in early differentiation stage in mouse embryonic stem cell-derived cardiomyocytes (mESC-CMs), but translocates from membrane to the sarcomere during the maturation of mESC-CMs. Translocation of TRPC7 occurs during the whole process of maturation; most channels locate near the M-line in less mature mESC-CMs while locate near the Z-line in more mature mESC-CMs. Lentivirus encoding specific shRNA was used to knockdown TRPC7 in neonatal rat cardiomyocytes. Immunocytochemistry staining demonstrated the decline of the TRPC7 expression, which was accompanied, intriguingly, with the disassembly of sarcomere. We speculated that both the full-length-TRPC7 and the truncated-TRPC7 (which lacking the transmembrane pore region) may exist in cardiomyocytes. The full-length-TRPC7, with its ion channel function, may participate in the regulation of electrophysiological function of cardiomyocytes in early differentiation stage, while the truncated-TRPC7 may insert into sarcomere to facilitate the stability of sarcomere.

## MicroRNAs regulate the sesquiterpenoid hormonal pathway in *Drosophila* and other arthropods

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Arthropods comprise the majority of all described animal species, and understanding their evolution is a central question in biology. Their developmental processes are under the precise control of distinct hormonal regulators, including the sesquiterpenoids juvenile hormone (JH) and methyl farnesoate (MF). The control of the synthesis and mode of action of these hormones play important roles in the evolution of arthropod biology and adaptation to diverse habitats. However, the precise roles of noncoding RNAs, such as microRNAs, controlling arthropod hormonal pathways are unknown. Here, we investigated the microRNA regulation of the expression of the juvenile hormone acid methyltransferase gene (*JHAMT*), which encodes a rate determining sesquiterpenoid biosynthetic enzyme. Loss-of-function of microRNA *bantam* in the fly *Drosophila melanogaster* increased *JHAMT* expression, while overexpression of the *bantam* repressed *JHAMT* expression and resulted in pupal lethality. The male genital organs of the pupae were malformed, and exogenous sesquiterpenoid application partially rescued the genital deformities. The role of *bantam* on the regulation of sesquiterpenoid biosynthesis was validated by transcriptomic, qPCR, and hormone titer (JHB3 and JH III) analyses. In addition, we found a conserved set of microRNAs that interacted with *JHAMT*, and the sesquiterpenoid receptor Methoprene-tolerant (*Met*) in different arthropod lineages, including insects (fly, mosquito and beetle), crustaceans (water flea and shrimp), myriapod (centipede) and chelicerate (horseshoe crab). This suggests that these microRNAs might have roles in the post-transcriptional regulation of genes in sesquiterpenoid pathways in the arthropod ancestor, rather than independently recruited. Some of the identified lineage-specific microRNAs are potential targets for the development of new strategies in aquaculture and agricultural pest control.

## Genome-wide analysis of trimethylation of histone H3 lysine 4 and acetylation of H3 lysine 9 in mature nodule

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Soybean could form nitrogen fixing nodules on root through the interaction with rhizobium, which is a unique feature of legumes. Nodule is a special organ differentiated from root via signals provided by the symbiotic relationship.

We hypothesize that the formation of this unique organ will involve a massive reprogramming of transcriptome in nodules, in which differential histone modification may play a key role. Histone modifications in H3 are reported to have function in many plant mechanisms and organ developments. Besides, the importance of histone modification also has been demonstrated in plant-microbe interactions.

We have found two differences on the global H3 modification level between remaining root and nodule revealing the global regulation of histone modifications in nodule. Based on the impact of histone modification on chromosome structure and gene expression, we have also performed the ChIP-Seq experiments on two histone modifications both in mature nodule and stripped root and analyzed the ChIP-Seq raw data. A general overview such as sequence quality, the consistency of two biological replicates, the ratio of unique mapping reads and peak annotation are obtained. From the ChIP-Seq data and the analysis of the target genes of H3K4me3 and H3K9ac implies the relationship of nodule function with histone modifications.

## Comparative analysis of DNA methylome dynamics during fruit ripening among eleven plants

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Fruit ripening is a biological process evolved to enhance seed dispersal in fleshy fruit-bearing species, and has contribute an important portion of animal and human diets. In the fruit model system tomato, DNA methylation was shown to be involved in this process. During ripening, CG cytosine went through a genome wide demethylation, and promoters of key ethylene biosynthesis genes like *ACS* and transcription factors like *MADS-RIN* and *CNR* are specifically demethylated. In addition, RNAi knockdown of DNA demethylase *SIDML2* inhibits ripening via hypermethylation and repression of those key ripening genes. However, it is unclear whether DNA demethylation occurs and target similar ripening genes in other fleshy fruit bearing plants.

In this study, we use whole genome bisulfite sequencing (BS-Seq) experiments to profile methylome of leaf, immature and ripe fruit of eleven species. We called differentially methylated region (DMR) between immature and found that, in all the studied plants, CG DMR were dominantly demethylated during ripening while methylation of CHH DMR was elevated. The hypomethylation of CG and hypermethylation of CHH exhibit in both gene region and transposon. Demethylated DMR were enriched in putative promoter of genes, indicating a potential role of transcription regulation. However, demethylation of ripening genes like *RIN* and *ACS* seems to be unique to tomato, suggesting a diversified epigenetic targeting scenario among those species. Further in-depth analysis is still ongoing. A web interface was set up to visualize all of the data generated in this study (<http://137.189.43.55/index.jsp>).

By profiling and featuring the DNA methylome in a wide-range of plant species, this project could provide a valuable insight into the molecular mechanism of fruit ripening with a unique evolutionary and epigenome perspective.

## Proteomic and peptidomic investigation on xylem sap to understand soybean salinity stress responses

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The xylem in plants has traditionally been considered to transport water, nutrients and metabolites, but recent evidences has suggested the functional role of xylem sap constituents mediating long-distance signaling in response to pathogens, nodulations and environmental stresses. Proteomics studies have revealed that xylem sap contains proteins involved in metabolisms, stress responses and signaling transductions. Noticeably, xylem mobile secreted peptides have been revealed as long-distance signaling molecules in response to surrounding changes. Up till now, only CLE-RS and CEP peptides induced by rhizobial inoculation and nitrogen starvation respectively have been studied. Recent studies support the hypothesis that vascular plants utilize xylem sap proteins and secreted peptides as organ-to-organ communication strategies in response to environmental change. This presentation aims to introduce the often-overlooked plant xylem sap proteomic and peptidomic as well as the potential in understanding the salinity stress response in plant via xylem sap.

## Time-series Analysis of Soybean Transcriptomes for the Identification of Novel Long Non-coding RNA Genes upon Salinity Stress

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Long non-coding RNAs (lncRNAs) are generally defined as non-protein coding transcripts which are less than 200 nucleotides and do not possess any ORF longer than 100 amino acid. They are known to play pivotal roles at transcriptional, post-transcriptional and epigenetic levels. Many lncRNA studies have focused on human and model organisms such as mouse and Arabidopsis, a comprehensive discovery of lncRNAs in soybean, however, is still absent at the moment.

We have made use of a set of RNA-seq data in order to identify lncRNAs in soybean. Samples in the RNA-seq experiment included both leaf and root tissues of cultivar, C08, and wild, W05, soybean germplasms across a series of time points with salt treatment (0.9% NaCl). In total, there are 824 lncRNA genes identified from the data set. Using time-series differential gene expression analysis, 352 lncRNA genes are found responsive to salt, of which 83 are induced by salt treatment at early time points (within 1 or 2 hours upon salt treatment) in root tissues. Gene ontology (GO) analysis has showed that calcium ion binding and oxidoreductase activity are significantly enriched in the nearest protein coding genes of the 83 lncRNA locus. Transposable elements (TEs) may be one of the factors driving the difference in lncRNA gene locus between C08 and W05. As for interspecies comparison, some of these lncRNA gene locus are conserved among other legume species, including *Lotus japonicus*, *Medicago truncatula* and *Phaseolus vulgaris*, to different extent. These evidence have led us to characterize one of the lncRNA locus, XLOC\_008250, and showed that it may be involved in salt stress regulation by cooperating with its upstream respiratory burst oxidase homolog D (RBOHD).

By complementing the with RNA-seq data with biologically related sets of mass spectrum (MS) data, we have shown that around 1% of the total identified spectra are encoded by 594 soybean lncRNAs. Some of these sORFs are well conserved in other legume species and potential to participate in salt stress regulations.

This study has suggested potential roles of lncRNA gene in stress regulations in soybean. Further functional characterizations are needed to unravel the detailed mechanisms of soybean lncRNA genes.

## Microscopic study of brassinosteroid-regulated chloroplast development in *Arabidopsis*

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Chloroplasts are essential organelles which dedicate to undergo photosynthesis to provide energy for plant survival and development. Normal biogenesis and development of chloroplasts ensure plants to obtain optimal amount of energy from photosynthesis. As a group of essential phytohormones, brassinosteroids (BRs) participate in many aspects of plant growth and development, including cell division, cell elongation, flowering, seed germination, and photosynthesis. However, little is known about the function of BRs in chloroplast development in plants. Previous studies in our laboratory have discovered a novel albino mutant *bzs20* in *Arabidopsis thaliana* from a BR related mutant screen. *bzs20* is a recessive T-DNA insertion mutant which is seedling lethal and shows albino cotyledons and pale green true leaves. Thus, *bzs20* mutation might have **caused** some defects in chloroplast development or synthesis of the green pigment chlorophyll. Besides, the dark green leaves of different BR-deficient and insensitive mutants imply that BRs may play a role in chloroplast development. To understand how the *bzs20* and BR related mutants affect chloroplast development, we are carrying out detailed phenotypic studies to the mutants, particularly chloroplast ultrastructure during seedling development using different approaches and methodologies. Ultrastructural analysis with TEM reveals that the *bzs20* mutant is destroyed in thylakoid network and lack of starch grains in mature chloroplast. However, before exposure to light etioplasts remain intact. Phenotypes of *bzs20* embryos during embryogenesis have been performed with light microscopy and no significant morphological difference was observed between *bzs20* and wild-type embryos, indicating that the albino phenotype is not formed during embryogenesis. Therefore, the albino phenotype is likely formed during seedling greening. These structural studies of chloroplast development in this novel albino mutant and BR mutants will help understand both the function of brassinosteroids in regulating chloroplast development and the mechanisms of chloroplast development in plants in general.

## Comparative Genomics of Marine and Terrestrial Flavobacteria

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Members of Flavobacteria are known as specialists for the degradation of detrital polysaccharides and peptides. They carry a considerable amount of genomic diversity and take a variety of lifestyles. Here, we performed comparative genomic analyses of over 170 strains sampled from marine and terrestrial environments. Phylogenomic analysis partitions all strains into five major lineages, including two marine-specific clades and two terrestrial-specific clades. Many metabolic pathways including the glycoside hydrolases for polysaccharide degradation and peptidases for peptide degradation are differentially distributed among clades, matching well with the chemistry of the ecological niches found in these distinct environments.

## A distinct class of vesicles derived from the *trans*-Golgi mediates secretion of xylogalacturonan in the root border cell

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Root border cells lie on the surface of the root cap and secrete massive amounts of mucilage that contains polysaccharides and proteoglycans. Golgi stacks in the border cells have hypertrophied margins, reflecting biosynthetic activity to produce polysaccharide components of the mucilage. To investigate the three-dimensional structures and macromolecular compositions of these Golgi stacks, we examined high-pressure frozen/freeze-substituted root cap cells with electron microscopy/tomography. Golgi stacks in border cells and peripheral cells displayed similar morphological features such as proliferation of trans cisternae and swellings in the trans cisternae and trans-Golgi network (TGN) compartments. These swellings give rise to two types of vesicles larger than other Golgi-associated vesicles. Swellings of trans-Golgi cisternae between medial and trans-most cisternae accumulate xylogalacturonan (XGA), and they become darkly stained large vesicles (LVs) after release from the Golgi. Xyloglucan (XG), polygalacturonic acid/rhamnogalacturonan-I (PGA/RG-I) are detected in the trans-most cisternae and TGN compartments. Large vesicles produced from TGN compartments (TGN-LVs) are lightly stained and carry XG and PGA/RG-I. XGA-carrying LVs fuse with the plasma membrane only in border cells, whereas TGN-LVs containing XG and PGA/RG-I fuse with the plasma membrane of both peripheral cells and border cells. Our results indicate that XGA is secreted by a novel type of secretory vesicles derived from trans-Golgi cisternae.

## Mining deep structure in multidimensional biological datasets with neural network

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There are two fundamental difficulties in biological data mining. First, biological dataset is often of small sample size and high-dimensional descriptive attributes, where a lot of noise exists. Second, the mechanism behind the datasets is always complex and difficult for data mining. In this study, we use the proposed multi-level feature selection method based on neural network for biological datasets with this kind of properties, such as metabolomics dataset to identifies metabolite markers, as well as the DNA microarray dataset, to understand the biological processes that underlie disease pathways.

The identification of target proteins interacting with drugs is one of the critical tasks in drug discovery. Experimental identification of targets is very challenging, so computational methods is necessary to identify drug target interactions. Here, we used the proposed multi-level feature selection method to identify activators of HIV-1 Integrase multimerization. It was indicated from the results that the prediction accuracy was rather promising, compared with that of docking simulation. Then, A TR-FRET-based biochemical high throughput dose response counterscreen assay was done for further experimental validation of the proposed feature selection method.

## Functional Characterization of A Plant U Box type E3 Ligase in Plant Immunity

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The U-box is a very conserved domain initially identified in yeast protein in charge of ubiquitination reaction. In *Arabidopsis* genome, there are more than 60 U-box containing proteins named Plant U-Box (PUB). Previously PUB4 together with its closest homologue PUB2 was identified as the *Arabidopsis* non-canonical G-protein XLG2-interacting proteins. The *pub4* single mutant and *pub2/4* double mutant displayed many similar phenotype as *xlg* mutants, indicating these genes play important roles in plant development and stress responses. XLG2/3 were reported to be phosphorylated by BIK1 and contribute to PTI. Here, we show that PUB4 could also interact with two key components in pathogen associated molecular pattern (PAMP)-triggered immunity (PTI), BIK1 and RbohD. The *pub2/4* mutant, like *xlg* mutants, exhibited compromised ROS burst in PTI. We are testing the possible mechanism that PUB4 directly ubiquitinate BIK1 or RbohD in defense response. Our study will provide new insights into the regulation mechanism of ROS production in plants.

## Effect of specific inhibitor of 3-hydroxy-3-methylglutaryl-CoA synthase (F-244) on Arabidopsis

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Isoprenoid compounds including chlorophylls and carotenoids, cytokinins, sterols, brassinosteroids, precursors of abscisic acid, monoterpenes and sesquiterpenes, promote the plant in many physiological processes and its survival. 3-Hydroxy-3-methylglutaryl-CoA synthase (HMGS) is an enzyme responsible for the condensation of acetoacetyl-CoA and acetyl-CoA into HMG-CoA, which involves in the second step in the mevalonate (MVA) biosynthesis pathway. MVA would subsequently be converted into IPP, a universal precursor for various isoprenoids. In plants, emerging studies show that the overexpression of HMGS in plants could significantly enhance germination, reproduction, seed production and stress tolerance, demonstrating that HMGS could be a potential target for manipulating plant growth regulation. Some of the most extensively-studied HMGS are the *Brassica juncea* BjHMGS1 and its mutant forms. Transgenic Arabidopsis and tobacco overexpressing BjHMGS1 and its mutant forms as well as the Arabidopsis knock-out mutant have been used to understand how HMGS affects physiological functions, downstream isoprenoid gene expression and end-product sterol production. In this study, to better understand the function of HMGS in plant development, an HMGS inhibitor was used to investigate if the metabolic flux from cytosolic acetyl-CoA to isoprenoids could be abrogated in Arabidopsis seedlings. Results on phenotypic changes arising from inhibitor use and analyses using qRT-PCR and proteomics will be presented.