Establishment of C4 Photosynthesis in the Maize *(Zea mays)* Cotyledon Examined by Electron Tomography

Peng Gao, Byung-Ho Kang
The Chinese University of Hong Kong, Hong Kong

Chloroplasts are the organelles responsible for photosynthesis upon which survival of all life forms depends. The chloroplast belongs to the organelle family termed plastids of which members interconvert according to genetic programs or by environmental cues. Chloroplasts arise from proplastids or etioplasts. Proplastids in the seed that germinated on the ground develop directly into chloroplasts. If germinated underground, seeds will convert proplastids into etioplasts which will transform into chloroplast later induced by light.

Classification of C4 and C3 plant species is based on differences in their mechanisms of photosynthesis. In most C4 grasses, such as maize *(Zea mays),* the photosynthetic apparatus is partitioned over two cell types, the mesophyll cells (MCs) and bundle sheath cells (BSCs) surrounding the vascular bundle. MCs capture carbon dioxide (CO2) from the atmosphere to produce four carbon molecules while BSCs run the Calvin cycle to produce carbohydrate using the four carbon molecules. The differentiation of MCs and BSCs in maize called Kranz anatomy is critical for suppressing oxygenase activity of Rubisco.

Maize is an economically valuable and well-characterized crop plant. Its C4 photosynthesis is an NADP-dependent maleic enzyme (NADP-ME) type in which MCs synthesize maleic acid that is transported into BSCs where it releases CO2 and produces NADPH. Chloroplasts in the two cell types of the maize monocotyledon are structurally and functionally distinct. By contrast, immature chloroplasts at earlier cotyledon germination stage do not exhibit such differentiation. The bifurcated pathway of the chloroplasts development in the maize cotyledon is of interest in the field of plant cell biology as well as crop engineering.

Electron tomography (ET) captures high-resolution images of cells or macromolecular complexes in three-dimension. When combined with high-pressure freezing (HPF) and ET can reveal mechanisms of organelle remodeling such as Golgi differentiation and mitochondrial fusion/fission dynamics. I propose to elucidate the process of dimorphic chloroplast biogenesis in the MCs and BSCs and assembly of thylakoid membrane of maize monocotyledon during germination using the advanced electron microscopy.

Majeran W.; Cai Y.; Sun Q.; van Wijk K.J. 2005. Functional differentiation of bundle sheath and mesophyll maize chloroplasts determined by comparative proteomics. Plant Cell 17:3111-3140.

Majeran, W.; Friso, G.; Ponnala, L.; Connolly, B.; Huang, M.; Reidel, E.; Van Wijk, K. 2010. Structural and metabolic transitions of C4 leaf development and differentiation defined by microscopy and quantitative proteomics in maize(W). Plant Cell 22:3509-3542.

Maurino V.G.; Drincovich M.F.; Casati P.; Andreo C.S.; Edwards G.E.; Ku M.S.B.; Gupta S.K.; Franceschi V.R. 1997. NADP-malic enzyme: Immunolocalization in different tissues of the C4 plant maize and the C3 plant wheat. Journal of Experimental Botany 48:799-811.

Paul J.; Enrique L.J. 2013. Biogenesis and homeostasis of chloroplasts and other plastids. Nature Reviews Molecular Cell Biology 14:787-802.

Pinghua Li.; Lalit P.; Neeru G.; Lin W.; Yaqing Si.; Tausta S. L.; Thomas P.B. 2010. The developmental dynamics of the maize leaf transcriptome. Nature Genetics 42:1060-1067.

Cryo-EM structure of human P-glycoprotein in the outward-facing conformation

Youngjin Kim and Jue Chen
The Chinese University of Hong Kong, Hong Kong

The Multidrug resistance to chemotherapeutics is a major obstacle to successful cancer treatment. ATP-binding cassette (ABC) transporter are often the culprits. These membrane bound transporter utilize the energy of ATP binding and hydrolysis to activity pump chemotherapy drugs from cells before they can reach their intracellular target, thus shielding the cells from the drug's cytotoxic effects. Several different ABC transporters have been implicated in the phenomenon including P-glycoprotein (P-gp), the breast cancer resistance protein (ABCG2), and the multidrug resistance protein (MRP1). P-glycoprotein extrudes toxic molecules and drugs from cells through ATP-powered conformational changes. Despite decades of effort, only the structures of the inward-facing conformation of P-glycoprotein are available.

To better understand the physiological roles of P-glycoprotein, we have solved high-resolution electron cryo-microscopy (cryo-EM) structure of human P-glycoprotein in the outward-facing conformation. The two nucleotide-binding domains form a closed dimer occluding two ATP molecules. The drug-binding cavity observed in the inward-facing structures is re-orientated toward the extracellular space and compressed to preclude substrate binding. Those features of structure elucidate the role of ATP in substrate release from the transporter. The structure evokes a model in which the dynamic nature of P-glycoprotein enables translocation of a large variety of substrates.

Youngjin Kim and Jue Chen (2018), Molecular structure of human P-glycoprotein in the ATP-bound, outward-facing conformation, Science 23 Feb 2018: Vol. 359, Issue 6378, pp. 915-919.

Three-Dimensional Structure of *Arabidopsis thaliana* ATG9

Louis Tung Faat Lai, Chuanyang Yu, Jan Siu Kei Wong, Ho Sing Lo, Samir Benlekbir, Liwen Jiang, Wilson Chun Yu Lau
The Chinese University of Hong Kong, Hong Kong SAR

Macroautophagy/autophagy is an essential process for the maintenance of cellular homeostasis by recycling macromolecules under normal and stress conditions. ATG9 (autophagy related 9) is the only integral membrane protein in the autophagy core machinery and has a central role in mediating autophagosome formation. In cells, ATG9 exists on mobile vesicles that traffic to the growing phagophore, providing an essential membrane source for the formation of autophagosomes. Here we report the three-dimensional structure of ATG9 from *Arabidopsis thaliana* at 7.8 Å resolution, determined by single particle cryo-electron microscopy. ATG9 organizes into a homotrimer, with each protomer contributing at least six transmembrane α-helices. At the center of the trimer, the protomers interact via their membrane-embedded and C-terminal cytoplasmic regions. Combined with prediction of protein contacts using sequence co-evolutionary information, the structure provides molecular insights into the ATG9 architecture and testable hypotheses for the molecular mechanism of autophagy progression regulated by ATG9.

Zhuang, X.; et al. 2017. ATG9 regulates autophagosome progression from the endoplasmic reticulum in Arabidopsis. Proc. Natl. Acad. Sci. USA 114(3):E426-435.

Papinski, D.; Schuschnig, M.; et al. 2014. Early steps in autophagy depend on direct phosphorylation of Atg9 by the Atg1 kinase. Molecular cell 53(3):471-483.

He, C.; Baba, M.; et al. 2008. Self-interaction is critical for Atg9 transport and function at the phagophore assembly site during autophagy. Mol. Biol. Cell 19(12):5506-5516.

He, C.; Baba, M. and Klionsky, D.J. 2009. Double duty of Atg9 self-association in autophagsome biogenesis. Autophagy 5(3):385-7.

Denisov, I. G., and Sligar, S. G. 2016. Nanodiscs for structural and functional studies of membrane proteins. Nature structural & molecular biology, 23(6): 481.

A Giant COPII Vesicle Population Orchestrating Plant Hormone Responses

Baiying Li, Yonglun Zeng, Wenhan Cao, Wenxin, Zhang, Lixin Cheng, Haidi Yin, Qian Wu, Xiangfeng Wang and Yan Huang
The Chinese University of Hong Kong, Hong Kong SAR

Higher plants live as sessile organisms with large-scale gene duplication events and subsequent paralog divergence during evolution. Notably, plant paralogs are expressed tissue-specifically and fine-tuned by phytohormones during various developmental processes. The coat protein complex II (COPII) is a highly conserved vesiculating machinery mediating protein transport from the Endoplasmic Reticulum (ER) to Golgi apparatus in eukaryotes. Intriguingly, plant COPII paralogs greatly outnumber those in yeast and mammals. However, the functional diversity and underlying mechanism of distinct COPII paralogs in regulating protein ER export and coping with various adverse environmental stresses are poorly understood. Here, we characterized a novel population of COPII vesicles produced in response to abscisic acid (ABA), a key phytohormone regulating abiotic stress responses in plants. These hormone-induced giant COPII vesicles are regulated by a plant specific COPII paralog and carry stress-related channels/transporters for alleviating stresses. This study thus provides a new mechanism underlying ABA-induced stress responses via the giant COPII vesicles, and answers a long-standing question of the evolutionary significance of gene duplications in higher plants.

Cryo-ET Analysis of the Yeast Synaptonemal Complex *In-Situ*

Olivia X. Ma, Shujun Cai, Jian Shi and Lu Gan
National University of Singapore, Singapore

The synaptonemal complex (SC) is the large proteinaceous scaffold that assembles between homologous chromosomes by the end of meiotic prophase. While its functions are numerous -- and mysterious, in yeast the SC is required for the phenomenon known as crossover interference. Knowledge of the native structure of this complex is needed to evaluate how the SC carries out its functions. Traditional electron microscopy and super-resolution light microscopy have revealed that in many organisms, the SC has a ladder-like structure: two rail-like lateral elements are bridged by a set of rung-like transverse filaments. The filaments are connected along their centers by a central element. To determine the 3-D architecture of the SC and nuclear macromolecular complexes in situ, we studied frozen-hydrated meiotic yeast cell cryosections by Volta electron cryotomography and subtomogram analysis. We find the predominant SC motif is a duster-like arrangement of densely packed triple-helical filaments, both of which are also abundant in the polycomplexes of pachytene-arrested cells. There was no evidence for a ladder-like organization. Partial dissolution by 1,6-hexanediol treatment suggests that these triple-helical filaments belong to the central region of the SCs and are most likely the abundant Zip1 transverse element protein. Subtomogram averaging revealed that the SC's triple helix is up to 12-nm thick and has a rise of 5 nm and a pitch of 130 nm. Polymers thinner than the triple helix, such as single or double strands, were not detected; this observation is consistent with the strong self-oligomerization properties of SC proteins. The dense packing of SC subunits supports the notion that the SC's mechanical properties help coordinate the rapid end-to-end communication across synapsed chromosomes. Finally, our study provides a 3-D framework for understanding the other macromolecule machines of meiosis in situ.

Single Particle Cryo-EM of *Helicobacter pylori* Trimeric Urease

Yap Shing Nim, Jan Siu Kei Wong, Wilson Chun Yu Lau and Kam-Bo Wong
The Chinese University of Hong Kong, Hong Kong SAR

Urease, urea amidohydrolase, can be found in many organisms other than mammals. Most of the discovered ureases are nickel enzymes. It facilitates *Helicobacter pylori* colonization in stomach, likely through the production of acid-neutralizing ammonia. Urease maturation requires four accessory proteins, UreE, UreF, UreG and UreH. Metallochaperone UreE passes the nickel to UreG via the formation of $UreE_2G_2$ complex in the presence of GTP. Nickel-bound UreG dimer is thought to switch to the pre-formed complex $UreF_2H_2$/Urease. Upon GTP hydrolysis of UreG, the nickel may translocate to urease via a water tunnel in the $UreF_2H_2$ complex.

Native plant ureases have a hexameric structure, while most of the bacterial ureases are trimeric. *Helicobacter pylori* urease has a very special dodecameric structure. Despite the different quaternary structures, the ureases have high sequence homology and similar folding. Interestingly, a large population of *H. pylori* urease trimer co-exists with the dodecamer during protein purification. However, unlike other bacterial ureases, *H. pylori* urease trimer could not be activated. Pulldown assay indicated a differential binding with HisGST-UreF/UreH complex between *H. pylori* urease dodecamer and trimer. Without any sign of protein degradation, we hypothesize that there may be structural differences between urease trimer and dodecamer. The difference may affect both the tetramerization of the urease trimer and its interaction with UreH. Single particle cryo EM is employed to determine the structure of *H. pylori* trimeric urease.

Mechanism Study of SH3P2 in Plant Mitophagy

Kaike Ren and Xiaohong Zhuang
The Chinese University of Hong Kong, Hong Kong SAR

Mitophagy is a selective form of autophagy in which mitochondria are selectively targeted and degraded in response to certain signals. This process is essential for development regulation, stress resistance and intracellular homeostasis maintenance in eukaryotes. In mammals, the Parkin-mediated mitophagy pathway is well described. Parkin, an E3 ligase, is translocated to the outer mitochondrial membrane (OMM) of damaged mitochondria to activate the ubiquitination of a bulk of OMM proteins, which ultimately leads to the recruitment of the core autophagy proteins (ATGs) and mitophagy. In addition, it is shown that several OMM-localised receptors contain an AIM/LIR (ATG8 interacting motif/LC3- interacting region) to mediate the parkin-independent pathways, such as ATG32 in yeast and FUNDC1 in human.

In plant, however, the underlying pathways of mitophagy remain to be elucidated as several key mitophagy players, for instance, homologues of yeast ATG32 and human FUNDC1, have not been found in plant. Previously, we reported a BAR-domain protein SH3P2 interacts with ATG8 and functions in autophagosome formation in Arabidopsis thaliana. Our preliminary results suggested, under normal condition, SH3P2 displays a mitochondrial-like pattern. But during mitophagy, SH3P2 forms a cup-like structure to engulf mitochondria, suggesting a possible role in mitochondria homeostasis. In order to identify novel regulators interacting with SH3P2 to regulate the selectivity in mitophagy, here I will use the tandem affinity pull down methods to purify the SH3P2 complex and to study their functional roles in mitochondria dynamics and mitophagy.

Broda, M.; Millar, A.; Van Aken, O. Trends In Plant Science, 2018, 23(5), 434-450.
2. Lazarou, M; et al. Nature, 2015, 524, 309– 31.

Liu L; Feng D; Chen G; et al. Nat. Cell Biol., 2012, 14, 177–185.

Okamoto, K; et al. Dev. Cell, 2009, 17, 87–97

Zhuang, X.; et. al. The Plant Cell, 2013, 25(11), 4596-4615.

Trans-Golgi mediated secretion of xylogalacturonan is critical for border-like cell release from the root cap

Pengfei Wang, Zhongyuan Liu and Byung-Ho Kang
The Chinese University of Hong Kong, Hong Kong

Root border cells constitute the surface of the root cap and secret massive amounts of mucilage that contains polysaccharides and proteoglycans. Golgi stacks in the border cells have hypertrophied margins, reflecting elevated biosynthetic activity to produce the polysaccharide components of the mucilage. We have recently shown that XGA is sorted into a new type of large vesicles derived from the *trans*-Golgi, termed XGA-LVs. Interestingly, XGA-LVs accumulate in border cell precursors, but they do not fuse with the plasma membrane until the precursor cells mature into border cells. The *Arabidopsis* root cap does not produce genuine border cells, but it sheds sheets of surface cells termed border-like cells (BLCs) that remain attached after releasing. To better understand the XGA synthesis and secretion in BLCs of the model plant *Arabidopsis*, we investigated the Golgi stacks in BLCs of *Arabidopsis* using laser-scanning confocal and electron microscopy coupled to high-pressure freezing/freezing substitution. Our results demonstrated that Golgi stacks in BLCs of 14-day-old seedling root caps exhibited ultrastructural features resembles to what we observed in border cell Golgi stacks. Darkly stained hypertrophied peripheries were seen in *trans*-Golgi cisternae in the 14-day-old but not 5-day-old root cap cells. Furthermore, the Golgi stacks of 14-day-old cells are labelled by LM8, but those of the 5-day-old *Arabidopsis* seedlings are not. These suggest that *Arabidopsis* BLCs secret XGA in a developmental stage-dependent manner. Besides, our CLEM data clearly show that the LM8 puncta in immuno fluorescence images corresponded to Golgi stacks in TEM. And the production of XGA in hypertrophied *trans*-Golgi cisternae is not affected by secretory vesicles formation of TGN in pi4kβ1/β2 null mutant. We also identify some *Arabidopsis* mutant lines (*brn1brn2, rcpg* and *syp42syp43*) in which border-like cells release is defective, and we demonstrated that the production of XGA is affected in some of the mutants. However, it is not understood what cargoes of XGA-LVs are, how they are selected, and how the trafficking is controlled. Our recently data indicate that XGA-LVs carry a polysaccharide-degrading enzyme. We will determine amino acid sequences in the protein required for its sorting. To identify more XGA-LV cargo proteins, we will isolate vesicle fractions associated with XGA epitopes from the root cap samples and carry out proteomic analyses.

Structural Study of Human ATM under Oxidation Stress by Cryo-EM

Jan Siu Kei Wong, Liwen Jiang and Wilson Chun Yu Lau
The Chinese University of Hong Kong, Hong Kong SAR

Ataxia-telangiectasia mutated (ATM) kinase is a signaling protein responsible for activating the DNA double-stand breaks (DSBs) repair and oxidative stress. Functional loss mutations in ATM lead to Ataxia telangiectasia (AT), which is a multisystem disorder characterized by genome instability, cancer predisposition, progressive neurologic impairment and immune dysfunction. Oxidative stress comes from ROS produced by cellular respiration reactions which occurs every second in our bodies. Free radicals from ROS can damage components of cell even induce strand breaks in DNA. Once activated by oxidative stress in the cell, ATM forms a covalent dimer which phosphorylates numerous downstream substrates including the tumor suppressor p53 to initiate DNA repair and arrest cell cycle progression. Our current understanding of the fundamental mechanism of ATM function and molecular pathology of AT is limited by the lack of the high-resolution structure of the active form of enzyme.

Traditional structural methods have proven challenging for solving the structure of the intact ATM dimer, which has molecular weight of 700 kDa. The aim of this study is to determine the atomic structure of the oxidized ATM dimer and ATM/P53 complex by single-particle cryo-electron microscopy (cryo-EM) in order to allow for better understanding of its working mechanism under oxidative stress.

Syed, A.; Tainer, J.A. 2018. The MRE11–RAD50–NBS1 Complex Conducts the Orchestration of Damage Signaling and Outcomes to Stress in DNA Replication and Repair. The Annual Review of Biochemistry, 87:263-294

Kumar, V. et al. 2017. Homeostatic nuclear RAGE–ATM interaction is essential for efficient DNA repair. Nucleic Acids Research, 45:10595–10613

Williams, R. S. et al. 2008. Mre11 Dimers Coordinate DNA End Bridging and Nuclease Processing in Double-Strand-Break Repair. Cell, 135: 97–109

Lammens, K. et al. 2011. The Mre11:Rad50 Structure Shows an ATP-Dependent Molecular Clamp in DNA Double-Strand Break Repair. Cell, 145:54–66

Yatime, L. et al. 2016. The Structure of the RAGE:S100A6 Complex Reveals a Unique Mode of Homodimerization for S100 Proteins. Structure, 24:2043–2052

Functional Study of the Interaction between SRPK2 and HBV Core Protein and the Regulatory Role of SRPK2 in HBV Replication

Pak Hong Yip and Jacky Ngo
The Chinese University of Hong Kong

Hepatitis B Virus (HBV) infection is a global health issue that many carriers have to bear the risk of development of severe liver diseases, as result of chronic injury to liver. The HBV replication cycle has been extensively revised. As shown in the diagram, there are four major steps of the viral replication: viral entry, DNA replication, virion formation and secretion. The host cellular serine-arginine protein specific kinases (SRPK1 and 2) have been identified to phosphorylate the HBV core protein and such phosphorylation is important for pregenomic RNA (pgRNA) encapsidation during the viral nucleocapsid assembly. SRPK2 is highly homologous to SRPK1, with the same critical amino acids at the docking groove which is crucial for substrate binding. On the other hand, phosphorylation mechanism of SRPKs towards a typical substrate SR protein have been studied, but not HBV core protein. Thus, my project will focus on the phosphorylation mechanism of SRPK2 on HBV core protein (HBVcp) and, more importantly, the structural basis of the interaction between SRPK2 and HBV core protein. Moreover, the role of the SRPK2 on HBV replication in cultured cell will also be investigated.

Cryo-EM Studies of the Chloroplast Small Heat Shock Protein Hsp21

Chuanyang Yu, King Pong Leung, Man Chit Wong, Jan Siu Kei Wong, Lai Tung Faat, Ho Sing Lo, Liwen Jiang and Wilson Chun Yu Lau
The Chinese University of Hong Kong, Hong Kong SAR

Environmental stresses often lead to protein unfolding and the formation of cytotoxic aggregates that compromise cell survival. In the cell, proper folding of proteins is constantly monitored and maintained by the protein quality control system. One essential component of protein quality control is molecular chaperones that stabilize non-native protein conformations and facilitate the refolding of proteins. The small heat shock proteins (sHsps) are ubiquitous and diverse family of molecular chaperones present in all living organisms. While they bind to and sequester misfolding proteins to prevent their aggregation, they themselves do not promote substrate refolding, distinguishing them from other major chaperone families.

How do sHsps recognize a wide variety of unfolding substrate proteins? Currently, little is known about the structural organization of the sHsp-substrate complexes due to their inherent heterogeneity and lack of high-resolution information. This proposed work focuses on the chloroplastic Hsp21, a member of the organelle-localized sHsps unique to plants. In preliminary analysis we identified a natural substrate of Hsp21, deoxyxylulose 5-phosphate synthase, which catalyzes the synthesis of precursors to metabolites that function in photosynthesis, growth regulation and plant-environment interaction. To obtain insights into the regulation and determinants of substrate binding and specificity of Hsp21, we will use cryo-EM combined with mutagenesis to study the Hsp21 structure and its interaction with deoxyxylulose 5-phosphate synthase. The proposed study herein will improve our understanding of the molecular mechanism of protein aggregation suppression by sHsps. Because overexpression of Hsp21 in transgenic plants enhances tolerance towards heat stress, the outcome of this research will offer potential applications for agricultural biotechnology to develop stress-resistant crops.

Bernfur, K., Rutsdottir, G. and Emanuelsson, C. 2017. The chloroplast-localized small heat shock protein associates with the thylakoid membranes in heat-stressed plants. Protein Science, 26(9), 1773-1784.

Lambert, W., Koeck, P.J., Ahrman, E., Purhonen, P., Cheng, K., Elmlund, D., Hebert, H., Emanuelsson, C. 2011. Subunit arrangement in the dodecameric chloroplast small heat shock protein Hsp21. Protein Sciences, 20(2), 291-301.

Stengel, F., Baldwin, A.J., Painter, A.J., Jaya, N., Basha, E., Kay, L.E., Vierling, E., Robinson, C.V. and Benesch, J.L. 2010. Quaternary dynamics and plasticity underlie small heat shock protein chaperone function. Proceedings of the National Academy of Sciences, 107(5), 2007-2012.

Van Montfort, R.L., Basha, E., Friedrich, K.L., Slingsby, C. and Vierling, E., 2001. Crystal structure and assembly of a eukaryotic small heat shock protein. Nature structural and molecular biology, 8(12), p.1025.

A Plant Specific AtSar1D-AtRabD2a Nexus Modulates Autophagy Biogenesis in Abiotic Stress

Yonglun Zeng, Baiying Li, Changyang Ji, Cesi Deng, Lixin Cheng, Shuai Chen, Youshun Lin, Jinbo Shen, Kenneth C.P. Cheung, Kam Bo Wong and Liwen Jiang
The Chinese University of Hong Kong, Hong Kong SAR

Secretory proteins traffic from endoplasmic reticulum (ER) to Golgi via coat protein complex II (COPII) vesicles in eukaryotes. Intriguingly, during nutrient starvation, COPII machinery constructively act as a membrane source for autophagosome, a double membrane-bound organelle that maintains cellular homeostasis by recycling intermediate metabolites. In higher plants, essential roles of autophagy have been implicated in plant development and stress responses. Nonetheless, the membrane sources of autophagosomes, especially the dedication of COPII machinery to autophagic pathway and autophagosome biogenesis remains elusive in plants. Moreover, how the differential regulation of COPII machinery fulfills distinct cellular roles (conventional secretion or autophagy) in response to environmental cues during plant development remains under-investigated, albeit the novel regulation of specific COPII isoforms in response to environmental stress has been elucidated recently. Here, we uncover the mechanistic connections between ATG (Autophagy-related gene) machinery and specific COPII components using proteomic analysis. Notably, a specific Sar1 homologue AtSar1d exhibits distinct effects on Atg8 lipidation and YFP-Atg8 vacuolar degradation upon autophagic induction through a previously unrevealed mechanism. Consistently, AtSar1d mutants exhibit starvation related phenotypes. Using the *Arabidopsis* protoplast transient expression system as screening platform, we further identify that a plant unique Rab1/Ypt1 homologue AtRabD2a coordinates with AtSar1d to function as the molecular switch in mediating the COPII functions in autophagy pathway. Indeed, AtRabD2a is essential for bridging the COPII machinery with autophagy initiation complex and contribute to autophagosome formation in plants. Our study thus identified a plant specific axis regulating autophagosome biogenesis and provide evidences on the evolutionary importance of gene duplication in higher plants.

Structural Studies of Plant Extracellular ATP Receptor DORN1

Wenzin Zhang, Liwen Jiang and Wilson Chun Yu Lau
The Chinese University of Hong Kong, Hong Kong

Adenosine 5′-triphosphate (ATP) is an essential energy source to drive biochemical reactions in all living organisms. ATP can be released to the extracellular matrix and is served as a signal transmitter, referred to as extracellular ATP (eATP). In mammals, eATP can be perceived by P2-type purinoceptors on the plasma membrane, whose dysfunction may cause various diseases, such as rheumatoid arthritis and hypertension. A novel ATP-insensitive mutant *Does not Respond to Nucleotides 1* (DORN1) was previously identified in *Arabidopsis thaliana.* DORN1 plays an important role in response to plant pathogen defense through direct phosphorylation of the NADPH oxidase RBOHD, resulting in the production of reactive oxygen species and stomatal closure. DORN1 can also activate the intracellular signaling of the defense hormone jasmonate to maximize the defense responses. Despite the fact that DORN1 has been reported to be involved in many aspects of plant development including plant photosynthesis, water homeostasis and pathogen resistance, the molecular mechanisms of DORN1 activation are not known due to the lack of any structural knowledge for this protein family

Single-particle cryo-electron microscopy (cryo-EM) is a technique that is used to determine the structures of macromolecules at atomic resolution in their near-native conditions. Here, we aim to study the structures of the DORN1 alone and in complex with its substrates by using single-particle cryo-EM to shed light onto its underlying mechanisms in plant development and the regulation of plant defense responses.

Chen, D. , Cao, Y. , Li, H. , Kim, D. , Ahsan, N. , & Thelen, J. , et al. (2017). Extracellular atp elicits dorn1-mediated rbohd phosphorylation to regulate stomatal aperture. Nature Communications, 8(1), 2265.

Hefetz, A., Keeling, C. I., Winston, M. L., Slessor, K. N., Nielsen, J., Lanfear, R., ... Volatiles, P. (2014). Identification of a Plant Receptor for Extracellular ATP, Science, (January), 290–295.

Kasuya, G. , Yamaura, T. , Ma, X. B. , Nakamura, R. , & Nureki, O. . (2017). Structural insights into the competitive inhibition of the atp-gated p2x receptor channel. Nature Communications,8(1).

Tripathi, D. , Zhang, T. , Koo, A. J. , Stacey, G. , & Tanaka, K. . (2017). Extracellular atp acts on jasmonate signaling to reinforce plant defense. Plant Physiology, pp.01477.2017.