**1. Protein-protein interaction and gene co-expression maps of ARFs and Aux/IAAs in Arabidopsis.**Piya S, Shrestha SK, Binder B, Stewart CN, Hewezi T

The phytohormone auxin regulates nearly all aspects of plant growth and development. Based on the current model in Arabidopsis thaliana, Auxin/indole-3-acetic acid (Aux/IAA) proteins repress auxin-inducible genes by inhibiting auxin response transcription factors (ARFs). Experimental evidence suggests that heterodimerization between Aux/IAA and ARF proteins are related to their unique biological functions. The objective of this study was to generate the Aux/IAA-ARF protein-protein interaction map using full length sequences and locate the interacting protein pairs to specific gene co-expression networks in order to define tissue-specific responses of the Aux/IAA-ARF interactome. Pairwise interactions between 19 ARFs and 29 Aux/IAAs resulted in the identification of 213 specific interactions of which 79 interactions were previously unknown. The incorporation of co-expression profiles with protein-protein interaction data revealed a strong correlation of gene co-expression for 70% of the ARF-Aux/IAA interacting pairs in at least one tissue/organ, indicative of the biological significance of these interactions. Importantly, ARF4-8 and 19, which were found to interact with almost all Aux-Aux/IAA showed broad co-expression relationships with Aux/IAA genes, thus, formed the central hubs of the co-expression network. Our analyses provide new insights into the biological significance of ARF-Aux/IAA associations in the morphogenesis and development of various plant tissues and organs.

Front Plant Sci Jan. 08, 2015; 5(0);744 [PUBMED:25566309]

**2. Selective homo- and heteromer interactions between the multiple organellar RNA editing factor (MORF) proteins in Arabidopsis thaliana.**

Zehrmann A, Haertel B, Glass F, Bayer-Csaszar E, Obata T, Meyer E, Brennicke A, Takenaka M

RNA editing in plastids and mitochondria of flowering plants requires pentatricopeptide repeat proteins (PPR proteins) for site recognition and proteins of the multiple organellar RNA editing factor (MORF) family as cofactors. Two MORF proteins, MORF5 and MORF8, are dual-targeted to plastids and mitochondria; two are targeted to plastids, and five are targeted to mitochondria. Pulldown assays from Arabidopsis thaliana tissue culture extracts with the mitochondrial MORF1 and the plastid MORF2 proteins, respectively, both identify the dual-targeted MORF8 protein, showing that these complexes can assemble in the organelles. We have now determined the scope of potential interactions between the various MORF proteins by yeast two-hybrid, in vitro pulldown, and bimolecular fluorescence complementation assays. The resulting MORF-MORF interactome identifies specific heteromeric MORF protein interactions in plastids and in mitochondria. Heteromers are observed for MORF protein combinations affecting a common site, suggesting their functional relevance. Most MORF proteins also undergo homomeric interactions. Submolecular analysis of the MORF1 protein reveals that the MORF-MORF protein connections require the C-terminal region of the central conserved MORF box. This domain has no similarity to known protein modules and may form a novel surface for protein-protein interactions.

J. Biol. Chem. Mar. 06, 2015; 290(10);6445-56 [PUBMED:25583991]

**3. The pentameric nucleoplasmin fold is present in Drosophila FKBP39 and a large number of chromatin-related proteins.**Edlich-Muth C, Artero JB, Callow P, Przewloka MR, Watson AA, Zhang W, Glover DM, Debski J, Dadlez M, Round AR, Trevor Forsyth V, Laue ED

Nucleoplasmin is a histone chaperone that consists of a pentameric N-terminal domain and an unstructured C-terminal tail. The pentameric core domain, a doughnut-like structure with a central pore, is only found in the nucleoplasmin family. Here, we report the first structure of a nucleoplasmin-like (NPL) domain from the unrelated Drosophila protein, FKBP39, and we present evidence that this protein associates with chromatin. Furthermore, we show that two other chromatin proteins, A. thaliana histone deacetylase type 2 (HD2) and S. cerevisiae Fpr4, share the NPL fold and form pentamers, in the case of HD2 a dimer of pentamers. Thus we propose a new family of proteins that share the pentameric nucleoplasmin-like NPL domain and are found in protists, fungi, plants and animals.

J. Mol. Biol. Mar. 23, 2015; 0(0); [PUBMED:25813344]

**4. Domains rearranged methyltransferase3 controls DNA methylation and regulates RNA polymerase V transcript abundance in Arabidopsis.**Zhong X, Hale CJ, Nguyen M, Ausin I, Groth M, Hetzel J, Vashisht AA, Henderson IR, Wohlschlegel JA, Jacobsen SE

DNA methylation is a mechanism of epigenetic gene regulation and genome defense conserved in many eukaryotic organisms. In Arabidopsis, the DNA methyltransferase domains rearranged methylase 2 (DRM2) controls RNA-directed DNA methylation in a pathway that also involves the plant-specific RNA Polymerase V (Pol V). Additionally, the Arabidopsis genome encodes an evolutionarily conserved but catalytically inactive DNA methyltransferase, DRM3. Here, we show that DRM3 has moderate effects on global DNA methylation and small RNA abundance and that DRM3 physically interacts with Pol V. In Arabidopsis drm3 mutants, we observe a lower level of Pol V-dependent noncoding RNA transcripts even though Pol V chromatin occupancy is increased at many sites in the genome. These findings suggest that DRM3 acts to promote Pol V transcriptional elongation or assist in the stabilization of Pol V transcripts. This work sheds further light on the mechanism by which long noncoding RNAs facilitate RNA-directed DNA methylation.

Mesh Terms:

Arabidopsis, DNA Methylation, DNA-Directed RNA Polymerases, Genes, Plant, Methyltransferases, RNA, Messenger

Proc. Natl. Acad. Sci. U.S.A. Jan. 20, 2015; 112(3);911-6 [PUBMED:25561521]

**5. Molecular characterization of the cold- and heat-induced Arabidopsis PXL1 gene and its potential role in transduction pathways under temperature fluctuations.**Jung CG, Hwang SG, Park YC, Park HM, Kim DS, Park DH, Jang CS

LRR-RLK (Leucine-Rich Repeat Receptor-Like Kinase) proteins are believed to play essential roles in cell-to-cell communication during various cellular processes including development, hormone perception, and abiotic stress responses. We isolated an LRR-RLK gene previously named Arabidopsis PHLOEM INTERCALATED WITH XYLEM-LIKE 1 (AtPXL1) and examined its expression patterns. AtPXL1 was highly induced by cold and heat stress, but not by drought. The fluorescence signal of 35S::AtPXL1-EGFP was closely localized to the plasma membrane. A yeast two-hybrid and bimolecular fluorescence complementation assay exhibited that AtPXL1 interacts with both proteins, A. thaliana histidine-rich dehydrin1 (AtHIRD1) and A. thaliana light-harvesting protein complex I (AtLHCA1). We found that AtPXL1 possesses autophosphorylation activity and phosphorylates AtHIRD1 and AtLHCA1 in an in vitro assay. Subsequently, we found that the knockout line (atpxl1) showed hypersensitive phenotypes when subjected to cold and heat during the germination stage, while the AtPXL1 overexpressing line as well as wild type plants showed high germination rates compared to the knockout plants. These results provide an insight into the molecular function of AtPXL1 in the regulation of signal transduction pathways under temperature fluctuations.

J. Plant Physiol. Mar. 15, 2015; 176(0);138-46 [PUBMED:25602612]

**6. The Ubiquitin Receptors DA1, DAR1, and DAR2 Redundantly Regulate Endoreduplication by Modulating the Stability of TCP14/15 in Arabidopsis.**

Peng Y, Chen L, Lu Y, Wu Y, Dumenil J, Zhu Z, Bevan MW, Li Y

Organ growth involves the coordination of cell proliferation and cell growth with differentiation. Endoreduplication is correlated with the onset of cell differentiation and with cell and organ size, but little is known about the molecular mechanisms linking cell and organ growth with endoreduplication. We have previously demonstrated that the ubiquitin receptor DA1 influences organ growth by restricting cell proliferation. Here, we show that DA1 and its close family members DAR1 and DAR2 are redundantly required for endoreduplication during leaf development. DA1, DAR1, and DAR2 physically interact with the transcription factors TCP14 and TCP15, which repress endoreduplication by directly regulating the expression of cell-cycle genes. We also show that DA1, DAR1, and DAR2 modulate the stability of TCP14 and TCP15 proteins in Arabidopsis thaliana. Genetic analyses demonstrate that DA1, DAR1, and DAR2 function in a common pathway with TCP14/15 to regulate endoreduplication. Thus, our findings define an important genetic and molecular mechanism involving the ubiquitin receptors DA1, DAR1, and DAR2 and the transcription factors TCP14 and TCP15 that links endoreduplication with cell and organ growth.

Plant Cell Mar. 01, 2015; 27(3);649-62 [PUBMED:25757472]

**7. TCP transcription factors are critical for the coordinated regulation of isochorismate synthase 1 expression in Arabidopsis thaliana.**Wang X, Gao J, Zhu Z, Dong X, Wang X, Ren G, Zhou X, Kuai B

Salicylic acid (SA) plays an important role in various aspects of plant development and responses to stresses. To elucidate the sophisticated regulatory mechanism of SA synthesis and signaling, we used a yeast one-hybrid system to screen for regulators of isochorismate synthase 1 (ICS1), a gene encoding the key enzyme in SA biosynthesis in Arabidopsis thaliana. A TCP family transcription factor AtTCP8 was initially identified as a candidate regulator of ICS1. The regulation of ICS1 by TCP proteins is supported by the presence of a typical TCP binding site in the ICS1 promoter. The binding of TCP8 to this site was confirmed by in vitro and in vivo assays. Expression patterns of TCP8 and its corresponding gene TCP9 largely overlapped with ICS1 under pathogen attack. A significant reduction in the expression of ICS1 during immune responses was observed in the tcp8 tcp9 double mutant. We also detected strong interactions between TCP8 and SAR deficient 1 (SARD1), WRKY family transcription factor 28 (WRKY28), NAC (NAM/ATAF1,ATAF2/CUC2) family transcription factor 019 (NAC019), as well as among TCP8, TCP9 and TCP20, suggesting a complex coordinated regulatory mechanism underlying ICS1 expression. Our results collectively demonstrate that TCP proteins are involved in the orchestrated regulation of ICS1 expression, with TCP8 and TCP9 being verified as major representatives.

Plant J. Apr. 01, 2015; 82(1);151-62 [PUBMED:25702611]

**8. A reversible Renilla luciferase protein complementation assay for rapid identification of protein-protein interactions reveals the existence of an interaction network involved in xyloglucan biosynthesis in the plant Golgi apparatus.**Lund CH, Bromley JR, Stenbaek A, Rasmussen RE, Scheller HV, Sakuragi Y

A growing body of evidence suggests that protein-protein interactions (PPIs) occur amongst glycosyltransferases (GTs) required for plant glycan biosynthesis (e.g. cell wall polysaccharides and N-glycans) in the Golgi apparatus, and may control the functions of these enzymes. However, identification of PPIs in the endomembrane system in a relatively fast and simple fashion is technically challenging, hampering the progress in understanding the functional coordination of the enzymes in Golgi glycan biosynthesis. To solve the challenges, we adapted and streamlined a reversible Renilla luciferase protein complementation assay (Rluc-PCA), originally reported for use in human cells, for transient expression in Nicotiana benthamiana. We tested Rluc-PCA and successfully identified luminescence complementation amongst Golgi-localizing GTs known to form a heterodimer (GAUT1 and GAUT7) and those which homooligomerize (ARAD1). In contrast, no interaction was shown between negative controls (e.g. GAUT7, ARAD1, IRX9). Rluc-PCA was used to investigate PPIs amongst Golgi-localizing GTs involved in biosynthesis of hemicelluloses. Although no PPI was identified among six GTs involved in xylan biosynthesis, Rluc-PCA confirmed three previously proposed interactions and identified seven novel PPIs amongst GTs involved in xyloglucan biosynthesis. Notably, three of the novel PPIs were confirmed by a yeast-based split-ubiquitin assay. Finally, Gateway-enabled expression vectors were generated, allowing rapid construction of fusion proteins to the Rluc reporters and epitope tags. Our results show that Rluc-PCA coupled with transient expression in N. benthamiana is a fast and versatile method suitable for analysis of PPIs between Golgi resident proteins in an easy and mid-throughput fashion in planta.

J. Exp. Bot. Jan. 01, 2015; 66(1);85-97 [PUBMED:25326916]

**9. Identification of Arabidopsis MYB56 as a novel substrate for CRL3(BPM) E3 ligases.**Chen L, Bernhardt A, Lee J, Hellmann H

Controlled stability of proteins is a highly efficient mechanism to direct diverse processes in living cells. A key regulatory system for protein stability is given by the ubiquitin proteasome pathway, which uses E3 ligases to mark specific proteins for degradation. In this work, MYB56 is identified as a novel target of a CULLIN3 (CUL3)-based E3 ligase. Its stability depends on the presence of MATH-BTB/POZ (BPM) proteins, which function as substrate adaptors to the E3 ligase. Genetic studies have indicated that MYB56 is a negative regulator of flowering, while BPMs positively affect this developmental program. The interaction between BPMs and MYB56 occurs at the promoter of FLOWERING LOCUS T (FT), a key regulator in initiating flowering in Arabidopsis, and results in instability of MYB56. Overall the work establishes MYB transcription factors as substrates of BPM proteins, and provides novel information on components that participate in controlling flowering time in plants.

Mol Plant Feb. 01, 2015; 8(2);242-50 [PUBMED:25618823]

**10. Three BUB1 and BUBR1/MAD3-related spindle assembly checkpoint proteins are required for accurate mitosis in Arabidopsis.**Paganelli L, Caillaud MC, Quentin M, Damiani I, Govetto B, Lecomte P,Karpov PA,Abad P,Chaboute ME, Favery B

The spindle assembly checkpoint (SAC) is a refined surveillance mechanism which ensures that chromosomes undergoing mitosis do not segregate until they are properly attached to the spindle microtubules (MT). The SAC has been extensively studied in metazoans and yeast, but little is known about its role in plants. We identified proteins interacting with a MT-associated protein MAP65-3, which plays a critical role in organising mitotic MT arrays, and carried out a functional analysis of previously and newly identified SAC components. We show that Arabidopsis SAC proteins BUB3.1, MAD2, BUBR1/MAD3s and BRK1 interact with each other and with MAP65-3. We found that two BUBR1/MAD3s interacted specifically at centromeres. When stably expressed in Arabidopsis, BRK1 localised to the kinetochores during all stages of the mitotic cell cycle. Early in mitosis, BUB3.1 and BUBR1/MAD3.1 localise to the mitotic spindle, where MAP65-3 organises spindle MTs. A double-knockout mad3.1 mad3.2 mutant presented spindle MT abnormalities, chromosome misalignments on the metaphase plate and the production of lagging chromosomes and micronuclei during mitosis. We conclude that BRK1 and BUBR1/MAD3-related proteins play a key role in ensuring faithful chromosome segregation during mitosis and that their interaction with MAP65-3 may be important for the regulation of MT-chromosome attachment.

New Phytol. Jan. 01, 2015; 205(1);202-15 [PUBMED:25262777]

**11. Arabidopsis dynamin-related proteins, DRP2A and DRP2B, function coordinately in post-Golgi trafficking.**Huang J, Fujimoto M, Fujiwara M, Fukao Y, Arimura S, Tsutsumi N

Dynamin-related proteins (DRPs) are large GTPases involved in a wide range of cellular membrane remodeling processes. In Arabidopsis thaliana, two paralogous land plant-specific type DRPs, DRP2A and DRP2B, are thought to participate in the regulation of post-Golgi trafficking. Here, we examined their molecular properties and functional relationships. qRT-PCR and GUS assays showed that DRP2A and DRP2B were expressed ubiquitously, although their expressions were strongest around root apical meristems and vascular bundles. Yeast two-hybrid, bi-molecular fluorescent complementation, and co-immunoprecipitation mass spectrometry analyses revealed that DRP2A and DRP2B interacted with each other. In observations with confocal laser scanning microscopy and variable incidence angle fluorescent microscopy, fluorescent fusions of DRP2A and DRP2B almost completely co-localized and were mainly localized to endocytic vesicle formation sites of the plasma membrane, clathrin-enriched trans-Golgi network and the cell plate in root epidermal cells. Treatments with wortmannin, an inhibitor of phosphatidylinositol 3-/4-kinases, latrunculin B, an inhibitor of actin polymerization, and oryzalin, an inhibitor of microtubule polymerization, increased the resident time of DRP2A and DRP2B on the plasma membrane. These results show that DRP2A and DRP2B function coordinately in multiple pathways of post-Golgi trafficking in phosphatidylinositol 3- or 4-kinase and cytoskeleton polymerization-dependent manners.

Mesh Terms: Actins, Androstadienes, Arabidopsis, Arabidopsis Proteins, Bicyclo Compounds, Heterocyclic, Cell Membrane, Cytoskeleton, Dinitrobenzenes, Dynamins, Enzyme Inhibitors, Fluorescent Dyes, GTP-Binding Proteins, Golgi Apparatus, Mass Spectrometry, Microscopy, Confocal, Microscopy, Fluorescence, Plant Roots, Protein Transport, Sulfanilamides, Thiazolidines, Two-Hybrid System Techniques

Biochem. Biophys. Res. Commun. Jan. 02, 2015; 456(1);238-44 [PUBMED:25462567]

**12. Iron-binding E3 ligase mediates iron response in plants by targeting basic helix-loop-helix transcription factors.**Selote D, Samira R, Matthiadis A, Gillikin JW, Long TA

Iron uptake and metabolism are tightly regulated in both plants and animals. In Arabidopsis (Arabidopsis thaliana), BRUTUS (BTS), which contains three hemerythrin (HHE) domains and a Really Interesting New Gene (RING) domain, interacts with basic helix-loop-helix transcription factors that are capable of forming heterodimers with POPEYE (PYE), a positive regulator of the iron deficiency response. BTS has been shown to have E3 ligase capacity and to play a role in root growth, rhizosphere acidification, and iron reductase activity in response to iron deprivation. To further characterize the function of this protein, we examined the expression pattern of recombinant ProBTS::β-GLUCURONIDASE and found that it is expressed in developing embryos and other reproductive tissues, corresponding with its apparent role in reproductive growth and development. Our findings also indicate that the interactions between BTS and PYE-like (PYEL) basic helix-loop-helix transcription factors occur within the nucleus and are dependent on the presence of the RING domain. We provide evidence that BTS facilitates 26S proteasome-mediated degradation of PYEL proteins in the absence of iron. We also determined that, upon binding iron at the HHE domains, BTS is destabilized and that this destabilization relies on specific residues within the HHE domains. This study reveals an important and unique mechanism for plant iron homeostasis whereby an E3 ubiquitin ligase may posttranslationally control components of the transcriptional regulatory network involved in the iron deficiency response.

Plant Physiol. Jan. 01, 2015; 167(1);273-86 [PUBMED:25452667]

**13. OST1 kinase modulates freezing tolerance by enhancing ICE1 stability in Arabidopsis.**Ding Y, Li H, Zhang X, Xie Q, Gong Z, Yang S

Cold stress is a major environmental factor that limits plant growth and development. The C-repeat-binding factor (CBF)-dependent cold signaling pathway is extensively studied in Arabidopsis; however, the specific protein kinases involved in this pathway remain elusive. Here we report that OST1 (open stomata 1), a well-known Ser/Thr protein kinase in ABA signaling, acts upstream of CBFs to positively regulate freezing tolerance. The ost1 mutants show freezing hypersensitivity, whereas transgenic plants overexpressing OST1 exhibit enhanced freezing tolerance. The OST1 kinase is activated by cold stress. Moreover, OST1 interacts with both the transcription factor ICE1 and the E3 ligase HOS1 in the CBF pathway. Cold-activated OST1 phosphorylates ICE1 and enhances its stability and transcriptional activity. Meanwhile, OST1 interferes with the interaction between HOS1 and ICE1, thus suppressing HOS1-mediated ICE1 degradation under cold stress. Our results thus uncover the unexpected roles of OST1 in modulating CBF-dependent cold signaling in Arabidopsis.

Dev. Cell Feb. 09, 2015; 32(3);278-89 [PUBMED:25669882]

**14. K-homology nuclear ribonucleoproteins regulate floral organ identity and determinacy in arabidopsis.**Rodriguez-Cazorla E, Ripoll JJ, Andujar A, Bailey LJ, Martinez-Laborda A, Yanofsky MF, Vera A

Post-transcriptional control is nowadays considered a main checking point for correct gene regulation during development, and RNA binding proteins actively participate in this process. Arabidopsis thaliana FLOWERING LOCUS WITH KH DOMAINS (FLK) and PEPPER (PEP) genes encode RNA-binding proteins that contain three K-homology (KH)-domain, the typical configuration of Poly(C)-binding ribonucleoproteins (PCBPs). We previously demonstrated that FLK and PEP interact to regulate FLOWERING LOCUS C (FLC), a central repressor of flowering time. Now we show that FLK and PEP also play an important role in the maintenance of the C-function during floral organ identity by post-transcriptionally regulating the MADS-box floral homeotic gene AGAMOUS (AG). Previous studies have indicated that the KH-domain containing protein HEN4, in concert with the CCCH-type RNA binding protein HUA1 and the RPR-type protein HUA2, facilitates maturation of the AG pre-mRNA. In this report we show that FLK and PEP genetically interact with HEN4, HUA1, and HUA2, and that the FLK and PEP proteins physically associate with HUA1 and HEN4. Taken together, these data suggest that HUA1, HEN4, PEP and FLK are components of the same post-transcriptional regulatory module that ensures normal processing of the AG pre-mRNA. Our data better delineates the roles of PEP in plant development and, for the first time, links FLK to a morphogenetic process.

PLoS Genet. Feb. 01, 2015; 11(2);e1004983 [PUBMED:25658099]

**15. An improved toolbox to unravel the plant cellular machinery by tandem affinity purification of Arabidopsis protein complexes.**Van Leene J, Eeckhout D, Cannoot B, De Winne N, Persiau G, Van De Slijke E, Vercruysse L, Dedecker M, Verkest A, Vandepoele K, Martens L, Witters E, Gevaert K, De Jaeger G

Tandem affinity purification coupled to mass spectrometry (TAP-MS) is one of the most advanced methods to characterize protein complexes in plants, giving a comprehensive view on the protein-protein interactions (PPIs) of a certain protein of interest (bait). The bait protein is fused to a double affinity tag, which consists of a protein G tag and a streptavidin-binding peptide separated by a very specific protease cleavage site, allowing highly specific protein complex isolation under near-physiological conditions. Implementation of this optimized TAP tag, combined with ultrasensitive MS, means that these experiments can be performed on small amounts (25 mg of total protein) of protein extracts from Arabidopsis cell suspension cultures. It is also possible to use this approach to isolate low abundant protein complexes from Arabidopsis seedlings, thus opening perspectives for the exploration of protein complexes in a plant developmental context. Next to protocols for efficient biomass generation of seedlings (∼7.5 months), we provide detailed protocols for TAP (1 d), and for sample preparation and liquid chromatography-tandem MS (LC-MS/MS; ∼5 d), either from Arabidopsis seedlings or from cell cultures. For the identification of specific co-purifying proteins, we use an extended protein database and filter against a list of nonspecific proteins on the basis of the occurrence of a co-purified protein among 543 TAP experiments. The value of the provided protocols is illustrated through numerous applications described in recent literature.

Nat Protoc Jan. 01, 2015; 10(1);169-87 [PUBMED:25521792]

**16. Increased phosphate transport of Arabidopsis thaliana Pht1;1 by site-directed mutagenesis of tyrosine 312 may be attributed to the disruption of homomeric interactions.**Fontenot EB, Ditusa SF, Kato N, Olivier DM, Dale R, Lin WY, Chiou TJ, Macnaughtan MA, Smith AP

Members of the Pht1 family of plant phosphate (Pi) transporters play vital roles in Pi acquisition from soil and in planta Pi translocation to maintain optimal growth and development. The study of the specificities and biochemical properties of Pht1 transporters will contribute to improving the current understanding of plant phosphorus homeostasis and use-efficiency. In this study, we show through split in vivo interaction methods and in vitro analysis of microsomal root tissues that Arabidopsis thaliana Pht1;1 and Pht1;4 form homomeric and heteromeric complexes. Transient and heterologous expression of the Pht1;1 variants, Pht1;1(Y312D) , Pht1;1(Y312A) and Pht1;1(Y312F) , was used to analyse the role of a putative Pi binding residue (Tyr 312) in Pht1;1 transporter oligomerization and function. The homomeric interaction among Pht1;1 proteins was disrupted by mutation of Tyr 312 to Asp, but not to Ala or Phe. In addition, the Pht1;1(Y312D) variant conferred enhanced Pi transport when expressed in yeast cells. In contrast, mutation of Tyr 312 to Ala or Phe did not affect Pht1;1 transport kinetics. Our study demonstrates that modifications to the Pht1;1 higher-order structure affects Pi transport, suggesting that oligomerization may serve as a regulatory mechanism for modulating Pi uptake.

Plant Cell Environ. Mar. 05, 2015; 0(0); [PUBMED:25754174]

**17. A Chaperone Function of NO CATALASE ACTIVITY1 Is Required to Maintain Catalase Activity and for Multiple Stress Responses in Arabidopsis.**Li J, Liu J, Wang G, Cha JY, Li G, Chen S, Li Z, Guo J, Zhang C, Yang Y, Kim WY, Yun DJ, Schumaker KS, Chen Z, Guo Y

Catalases are key regulators of reactive oxygen species homeostasis in plant cells. However, the regulation of catalase activity is not well understood. In this study, we isolated an Arabidopsis thaliana mutant, no catalase activity1-3 (nca1-3) that is hypersensitive to many abiotic stress treatments. The mutated gene was identified by map-based cloning as NCA1, which encodes a protein containing an N-terminal RING-finger domain and a C-terminal tetratricopeptide repeat-like helical domain. NCA1 interacts with and increases catalase activity maximally in a 240-kD complex in planta. In vitro, NCA1 interacts with CATALASE2 (CAT2) in a 1:1 molar ratio, and the NCA1 C terminus is essential for this interaction. CAT2 activity increased 10-fold in the presence of NCA1, and zinc ion binding of the NCA1 N terminus is required for this increase. NCA1 has chaperone protein activity that may maintain the folding of catalase in a functional state. NCA1 is a cytosol-located protein. Expression of NCA1 in the mitochondrion of the nca1-3 mutant does not rescue the abiotic stress phenotypes of the mutant, while expression in the cytosol or peroxisome does. Our results suggest that NCA1 is essential for catalase activity.

Plant Cell Mar. 01, 2015; 27(3);908-25 [PUBMED:25700484]

**18. A functional Small Ubiquitin-like Modifier (SUMO) interacting motif (SIM) in the gibberellin hormone receptor GID1 is conserved in cereal crops and disrupting this motif does not abolish hormone dependency of the DELLA-GID1 interaction.**Nelis S, Conti L, Zhang C, Sadanandom A

Plants survive adversity by modulating their growth in response to changing environmental signals. The phytohormone Gibberellic acid (GA) plays a central role in regulating these adaptive responses by stimulating the degradation of growth repressing DELLA proteins which accumulate during stress. The current model for GA signaling describes how this hormone binds to its receptor GID1 so promoting association of GID1 with DELLA, which then undergoes ubiquitin-mediated proteasomal degradation. Recent data revealed that conjugation of DELLAs to the Small Ubiquitin-like Modifier (SUMO) protein enables plants to modulate its abundance during environmental stress. This is achieved by SUMOylated DELLAs sequestering GID1 via its SUMO interacting motif (SIM) allowing non-SUMOylated DELLAs to accumulate leading to growth restraint under stress and potential yield loss. We demonstrate that GID1 proteins across the major cereal crops contain a functional SIM able to bind SUMO1. Site directed mutagenesis and yeast 2 hybrid experiments reveal that it is possible to disrupt the SIM-SUMO interaction motif without affecting the GA dependent DELLA-GID1 interaction and thereby uncoupling SUMO-mediated inhibition from DELLA degradation. Arabidopsis plants overexpressing a SIM mutant allele of GID1 perform better at relieving DELLA restraint than wild-type GID1. This evidence suggests that manipulating the SIM motif in the GA receptor may provide a possible route to developing stress tolerant crops plants.

Plant Signal Behav Feb. 01, 2015; 10(2);e987528 [PUBMED:25761145]

**19. Binding of SEC11 Indicates Its Role in SNARE Recycling after Vesicle Fusion and Identifies Two Pathways for Vesicular Traffic to the Plasma Membrane.**Karnik R, Zhang B, Waghmare S, Aderhold C, Grefen C, Blatt MR

SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins drive vesicle fusion in all eukaryotes and contribute to homeostasis, pathogen defense, cell expansion, and growth in plants. Two homologous SNAREs, SYP121 (=SYR1/PEN1) and SYP122, dominate secretory traffic to the Arabidopsis thaliana plasma membrane. Although these proteins overlap functionally, differences between SYP121 and SYP122 have surfaced, suggesting that they mark two discrete pathways for vesicular traffic. The SNAREs share primary cognate partners, which has made separating their respective control mechanisms difficult. Here, we show that the regulatory protein SEC11 (=KEULE) binds selectively with SYP121 to affect secretory traffic mediated by this SNARE. SEC11 rescued traffic block by dominant-negative (inhibitory) fragments of both SNAREs, but only in plants expressing the native SYP121. Traffic and its rescue were sensitive to mutations affecting SEC11 interaction with the N terminus of SYP121. Furthermore, the domain of SEC11 that bound the SYP121 N terminus was itself able to block secretory traffic in the wild type and syp122 but not in syp121 mutant Arabidopsis. Thus, SEC11 binds and selectively regulates secretory traffic mediated by SYP121 and is important for recycling of the SNARE and its cognate partners.

Plant Cell Mar. 01, 2015; 27(3);675-94 [PUBMED:25747882]

**20. Protein intrinsic disorder in Arabidopsis NAC transcription factors: transcriptional activation by ANAC013 and ANAC046 and their interactions with RCD1.**O'Shea C, Kryger M, Stender EG, Kragelund BB, Willemoes M, Skriver K

Protein ID (intrinsic disorder) plays a significant, yet relatively unexplored role in transcription factors (TFs). In the present paper, analysis of the transcription regulatory domains (TRDs) of six phylogenetically representative, plant-specific NAC [no apical meristem, ATAF (Arabidopsis transcription activation factor), cup-shaped cotyledon] TFs shows that the domains are present in similar average pre-molten or molten globule-like states, but have different patterns of order/disorder and MoRFs (molecular recognition features). ANAC046 (Arabidopsis NAC 046) was selected for further studies because of its simple MoRF pattern and its ability to interact with RCD1 (radical-induced cell death 1). Experiments in yeast and thermodynamic characterization suggest that its single MoRF region is sufficient for both transcriptional activation and interaction with RCD1. The remainder of the large regulatory domain is unlikely to contribute to the interaction, since the domain and truncations thereof have similar affinities for RCD1, which are also similar for ANAC013-RCD1 interactions. However, different enthalpic and entropic contributions to binding were revealed for ANAC046 and ANAC013, suggestive of differences in binding mechanisms. Although substitution of both hydrophobic and acidic residues of the ANAC046 MoRF region abolished binding, substitution of other residues, even with α-helix-breaking proline, was less disruptive. Together, the biophysical analyses suggest that RCD1-ANAC046 complex formation does not involve folding-upon-binding, but rather fuzziness or an unknown structure in ANAC046. We suggest that the ANAC046 regulatory domain functions as an entropic chain with a terminal hot spot interacting with RCD1. RCD1, a cellular hub, may be able to interact with many different TFs by exploiting their ID-based flexibility, as demonstrated for its interactions with ANAC046 and ANAC013.

Mesh Terms:

Arabidopsis, Arabidopsis Proteins, Gene Expression Regulation, Plant, Nuclear Proteins, Protein Binding, Protein Structure, Tertiary, Transcription Factors, Transcriptional Activation

Biochem. J. Jan. 15, 2015; 465(2);281-94 [PUBMED:25348421]

**21. Functional mapping of the plant small RNA methyltransferase: HEN1 physically interacts with HYL1 and DICER-LIKE 1 proteins.**Baranauske S, Mickute M, Plotnikova A, Finke A, Venclovas C, Klimasauskas S, Vilkaitis G

Methylation of 3'-terminal nucleotides of miRNA/miRNA\* is part of miRNAs biogenesis in plants but is not found in animals. In Arabidopsis thaliana this reaction is carried out by a multidomain AdoMet-dependent 2'-O-methyltransferase HEN1. Using deletion and structure-guided mutational analysis, we show that the double-stranded RNA-binding domains R(1) and R(2) of HEN1 make significant but uneven contributions to substrate RNA binding, and map residues in each domain responsible for this function. Using GST pull-down assays and yeast two-hybrid analysis we demonstrate direct HEN1 interactions, mediated by its FK506-binding protein-like domain and R(2) domain, with the microRNA biogenesis protein HYL1. Furthermore, we find that HEN1 forms a complex with DICER-LIKE 1 (DCL1) ribonuclease, another key protein involved in miRNA biogenesis machinery. In contrast, no direct interaction is detectable between HEN1 and SERRATE. On the basis of these findings, we propose a mechanism of plant miRNA maturation which involves binding of the HEN1 methyltransferase to the DCL1•HYL1•miRNA complex excluding the SERRATE protein.

Nucleic Acids Res. Mar. 11, 2015; 43(5);2802-12 [PUBMED:25680966]

**22. Light-activated phytochrome A and B interact with members of the SPA family to promote photomorphogenesis in Arabidopsis by reorganizing the COP1/SPA complex.**Sheerin DJ, Menon C, zur Oven-Krockhaus S, Enderle B, Zhu L, Johnen P, Schleifenbaum F, Stierhof YD, Huq E, Hiltbrunner A

Phytochromes function as red/far-red photoreceptors in plants and are essential for light-regulated growth and development. Photomorphogenesis, the developmental program in light, is the default program in seed plants. In dark-grown seedlings, photomorphogenic growth is suppressed by the action of the CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1)/SUPPRESSOR OF phyA-105 (SPA) complex, which targets positive regulators of photomorphogenic growth for degradation by the proteasome. Phytochromes inhibit the COP1/SPA complex, leading to the accumulation of transcription factors promoting photomorphogenesis; yet, the mechanism by which they inactivate COP1/SPA is still unknown. Here, we show that light-activated phytochrome A (phyA) and phytochrome B (phyB) interact with SPA1 and other SPA proteins. Fluorescence resonance energy transfer-fluorescence lifetime imaging microscopy analyses show that SPAs and phytochromes colocalize and interact in nuclear bodies. Furthermore, light-activated phyA and phyB disrupt the interaction between COP1 and SPAs, resulting in reorganization of the COP1/SPA complex in planta. The light-induced stabilization of HFR1, a photomorphogenic factor targeted for degradation by COP1/SPA, correlates temporally with the accumulation of phyA in the nucleus and localization of phyA to nuclear bodies. Overall, these data provide a molecular mechanism for the inactivation of the COP1/SPA complex by phyA- and phyB-mediated light perception.

Plant Cell Jan. 01, 2015; 27(1);189-201 [PUBMED:25627066]

**23. Arabidopsis AIP1-2 restricted by WER-mediated patterning modulates planar polarity.**Kiefer CS, Claes AR, Nzayisenga JC, Pietra S, Stanislas T, Hueser A, Ikeda Y, Grebe M

The coordination of cell polarity within the plane of the tissue layer (planar polarity) is crucial for the development of diverse multicellular organisms. Small Rac/Rho-family GTPases and the actin cytoskeleton contribute to planar polarity formation at sites of polarity establishment in animals and plants. Yet, upstream pathways coordinating planar polarity differ strikingly between kingdoms. In the root of Arabidopsis thaliana, a concentration gradient of the phytohormone auxin coordinates polar recruitment of Rho-of-plant (ROP) to sites of polar epidermal hair initiation. However, little is known about cytoskeletal components and interactions that contribute to this planar polarity or about their relation to the patterning machinery. Here, we show that ACTIN7 (ACT7) represents a main actin isoform required for planar polarity of root hair positioning, interacting with the negative modulator ACTIN-INTERACTING PROTEIN1-2 (AIP1-2). ACT7, AIP1-2 and their genetic interaction are required for coordinated planar polarity of ROP downstream of ethylene signalling. Strikingly, AIP1-2 displays hair cell file-enriched expression, restricted by WEREWOLF (WER)-dependent patterning and modified by ethylene and auxin action. Hence, our findings reveal AIP1-2, expressed under control of the WER-dependent patterning machinery and the ethylene signalling pathway, as a modulator of actin-mediated planar polarity.

Mesh Terms:

Actins, Arabidopsis, Arabidopsis Proteins, Body Patterning, Carrier Proteins, Cell Polarity, DNA-Binding Proteins, Epistasis, Genetic, Ethylenes, Plant Roots, Protein Binding, Saccharomyces cerevisiae, Signal Transduction

Development Jan. 01, 2015; 142(1);151-61 [PUBMED:25428588]

**24. Retromer contributes to immunity-associated cell death in Arabidopsis.**

Munch D, Teh OK, Malinovsky FG, Liu Q, Vetukuri RR, El Kasmi F, Brodersen P, Hara-Nishimura I, Dangl JL, Petersen M, Mundy J, Hofius D

Membrane trafficking is required during plant immune responses, but its contribution to the hypersensitive response (HR), a form of programmed cell death (PCD) associated with effector-triggered immunity, is not well understood. HR is induced by nucleotide binding-leucine-rich repeat (NB-LRR) immune receptors and can involve vacuole-mediated processes, including autophagy. We previously isolated lazarus (laz) suppressors of autoimmunity-triggered PCD in the Arabidopsis thaliana mutant accelerated cell death11 (acd11) and demonstrated that the cell death phenotype is due to ectopic activation of the LAZ5 NB-LRR. We report here that laz4 is mutated in one of three VACUOLAR PROTEIN SORTING35 (VPS35) genes. We verify that LAZ4/VPS35B is part of the retromer complex, which functions in endosomal protein sorting and vacuolar trafficking. We show that VPS35B acts in an endosomal trafficking pathway and plays a role in LAZ5-dependent acd11 cell death. Furthermore, we find that VPS35 homologs contribute to certain forms of NB-LRR protein-mediated autoimmunity as well as pathogen-triggered HR. Finally, we demonstrate that retromer deficiency causes defects in late endocytic/lytic compartments and impairs autophagy-associated vacuolar processes. Our findings indicate important roles of retromer-mediated trafficking during the HR; these may include endosomal sorting of immune components and targeting of vacuolar cargo.

Plant Cell Feb. 01, 2015; 27(2);463-79 [PUBMED:25681156]

**25. Protein poly(ADP-ribosyl)ation regulates arabidopsis immune gene expression and defense responses.**Feng B, Liu C, de Oliveira MV, Intorne AC, Li B, Babilonia K, de Souza Filho GA, Shan L, He P

Perception of microbe-associated molecular patterns (MAMPs) elicits transcriptional reprogramming in hosts and activates defense to pathogen attacks. The molecular mechanisms underlying plant pattern-triggered immunity remain elusive. A genetic screen identified Arabidopsis poly(ADP-ribose) glycohydrolase 1 (atparg1) mutant with elevated immune gene expression upon multiple MAMP and pathogen treatments. Poly(ADP-ribose) glycohydrolase (PARG) is predicted to remove poly(ADP-ribose) polymers on acceptor proteins modified by poly(ADP-ribose) polymerases (PARPs) with three PARPs and two PARGs in Arabidopsis genome. AtPARP1 and AtPARP2 possess poly(ADP-ribose) polymerase activity, and the activity of AtPARP2 was enhanced by MAMP treatment. AtPARG1, but not AtPARG2, carries glycohydrolase activity in vivo and in vitro. Importantly, mutation (G450R) in atparg1 blocks its activity and the corresponding residue is highly conserved and essential for human HsPARG activity. Consistently, mutant atparp1atparp2 plants exhibited compromised immune gene activation and enhanced susceptibility to pathogen infections. Our study indicates that protein poly(ADP-ribosyl)ation plays critical roles in plant immune gene expression and defense to pathogen attacks.

PLoS Genet. Jan. 01, 2015; 11(1);e1004936 [PUBMED:25569773]

**26. Plant nuclear shape is independently determined by the SUN-WIP-WIT2-myosin XI-i complex and CRWN1.**Zhou X, Groves NR, Meier I

Nuclei undergo dynamic shape changes during plant development, but the mechanism is unclear. In Arabidopsis, Sad1/UNC-84 (SUN) proteins, WPP domain-interacting proteins (WIPs), WPP domain-interacting tail-anchored proteins (WITs), myosin XI-i, and CROWDED NUCLEI 1 (CRWN1) have been shown to be essential for nuclear elongation in various epidermal cell types. It has been proposed that WITs serve as adaptors linking myosin XI-i to the SUN-WIP complex at the nuclear envelope (NE). Recently, an interaction between Arabidopsis SUN1 and SUN2 proteins and CRWN1, a plant analog of lamins, has been reported. Therefore, the CRWN1-SUN-WIP-WIT-myosin XI-i interaction may form a linker of the nucleoskeleton to the cytoskeleton complex. In this study, we investigate this proposed mechanism in detail for nuclei of Arabidopsis root hairs and trichomes. We show that WIT2, but not WIT1, plays an essential role in nuclear shape determination by recruiting myosin XI-i to the SUN-WIP NE bridges. Compared with SUN2, SUN1 plays a predominant role in nuclear shape. The NE localization of SUN1, SUN2, WIP1, and a truncated WIT2 does not depend on CRWN1. While crwn1 mutant nuclei are smooth, the nuclei of sun or wit mutants are invaginated, similar to the reported myosin XI-i mutant phenotype. Together, this indicates that the roles of the respective WIT and SUN paralogs have diverged in trichomes and root hairs, and that the SUN-WIP-WIT2-myosin XI-i complex and CRWN1 independently determine elongated nuclear shape. This supports a model of nuclei being shaped both by cytoplasmic forces transferred to the NE and by nucleoplasmic filaments formed under the NE.

Nucleus Mar. 04, 2015; 6(2);144-53 [PUBMED:25759303]

**27. TYPE-ONE PROTEIN PHOSPHATASE4 regulates pavement cell interdigitation by modulating PIN-FORMED1 polarity and trafficking in Arabidopsis.**Guo X, Qin Q, Yan J, Niu Y, Huang B, Guan L, Li Y, Ren D, Li J, Hou S

In plants, cell morphogenesis is dependent on intercellular auxin accumulation. The polar subcellular localization of the PIN-FORMED (PIN) protein is crucial for this process. Previous studies have shown that the protein kinase PINOID (PID) and protein phosphatase6-type phosphatase holoenzyme regulate the phosphorylation status of PIN1 in root tips and shoot apices. Here, we show that a type-one protein phosphatase, TOPP4, is essential for the formation of interdigitated pavement cell (PC) pattern in Arabidopsis (Arabidopsis thaliana) leaf. The dominant-negative mutant topp4-1 showed severely inhibited interdigitated PC growth. Expression of topp4-1 gene in wild-type plants recapitulated the PC defects in the mutant. Genetic analyses suggested that TOPP4 and PIN1 likely function in the same pathway to regulate PC morphogenesis. Furthermore, colocalization, in vitro and in vivo protein interaction studies, and dephosphorylation assays revealed that TOPP4 mediated PIN1 polar localization and endocytic trafficking in PCs by acting antagonistically with PID to modulate the phosphorylation status of PIN1. In addition, TOPP4 affects the cytoskeleton pattern through the Rho of Plant GTPase-dependent auxin-signaling pathway. Therefore, we conclude that TOPP4-regulated PIN1 polar targeting through direct dephosphorylation is crucial for PC morphogenesis in the Arabidopsis leaf.

Plant Physiol. Mar. 01, 2015; 167(3);1058-75 [PUBMED:25560878]

**28. NIK1-mediated translation suppression functions as a plant antiviral immunity mechanism.**Zorzatto C, Machado JP, Lopes KV, Nascimento KJ, Pereira WA, Brustolini OJ, Reis PA, Calil IP, Deguchi M, Sachetto-Martins G, Gouveia BC,Loriato VA, Silva MA, Silva FF, Santos AA, Chory J, Fontes EP

Plants and plant pathogens are subject to continuous co-evolutionary pressure for dominance, and the outcomes of these interactions can substantially impact agriculture and food security. In virus-plant interactions, one of the major mechanisms for plant antiviral immunity relies on RNA silencing, which is often suppressed by co-evolving virus suppressors, thus enhancing viral pathogenicity in susceptible hosts. In addition, plants use the nucleotide-binding and leucine-rich repeat (NB-LRR) domain-containing resistance proteins, which recognize viral effectors to activate effector-triggered immunity in a defence mechanism similar to that employed in non-viral infections. Unlike most eukaryotic organisms, plants are not known to activate mechanisms of host global translation suppression to fight viruses. Here we demonstrate in Arabidopsis that the constitutive activation of NIK1, a leucine-rich repeat receptor-like kinase (LRR-RLK) identified as a virulence target of the begomovirus nuclear shuttle protein (NSP), leads to global translation suppression and translocation of the downstream component RPL10 to the nucleus, where it interacts with a newly identified MYB-like protein, L10-INTERACTING MYB DOMAIN-CONTAINING PROTEIN (LIMYB), to downregulate translational machinery genes fully. LIMYB overexpression represses ribosomal protein genes at the transcriptional level, resulting in protein synthesis inhibition, decreased viral messenger RNA association with polysome fractions and enhanced tolerance to begomovirus. By contrast, the loss of LIMYB function releases the repression of translation-related genes and increases susceptibility to virus infection. Therefore, LIMYB links immune receptor LRR-RLK activation to global translation suppression as an antiviral immunity strategy in plants.

Nature Feb. 23, 2015; 0(0); [PUBMED:25707794]

**29. Recruitment of PLANT U-BOX13 and the PI4Kβ1/β2 phosphatidylinositol-4 kinases by the small GTPase RabA4B plays important roles during salicylic acid-mediated plant defense signaling in Arabidopsis.**Antignani V, Klocko AL, Bak G, Chandrasekaran SD, Dunivin T, Nielsen E

Protection against microbial pathogens involves the activation of cellular immune responses in eukaryotes, and this cellular immunity likely involves changes in subcellular membrane trafficking. In eukaryotes, members of the Rab GTPase family of small monomeric regulatory GTPases play prominent roles in the regulation of membrane trafficking. We previously showed that RabA4B is recruited to vesicles that emerge from trans-Golgi network (TGN) compartments and regulates polarized membrane trafficking in plant cells. As part of this regulation, RabA4B recruits the closely related phosphatidylinositol 4-kinase (PI4K) PI4Kβ1 and PI4Kβ2 lipid kinases. Here, we identify a second Arabidopsis thaliana RabA4B-interacting protein, PLANT U-BOX13 (PUB13), which has recently been identified to play important roles in salicylic acid (SA)-mediated defense signaling. We show that PUB13 interacts with RabA4B through N-terminal domains and with phosphatidylinositol 4-phosphate (PI-4P) through a C-terminal armadillo domain. Furthermore, we demonstrate that a functional fluorescent PUB13 fusion protein (YFP-PUB13) localizes to TGN and Golgi compartments and that PUB13, PI4Kβ1, and PI4Kβ2 are negative regulators of SA-mediated induction of pathogenesis-related gene expression. Taken together, these results highlight a role for RabA4B and PI-4P in SA-dependent defense responses.

Plant Cell Jan. 01, 2015; 27(1);243-61 [PUBMED:25634989]

**30. Overexpression of the PP2A regulatory subunit Tap46 leads to enhanced plant growth through stimulation of the TOR signalling pathway.**Ahn CS, Ahn HK, Pai HS

Tap46, a regulatory subunit of protein phosphatase 2A (PP2A), plays an essential role in plant growth and development through a functional link with the Target of Rapamycin (TOR) signalling pathway. Here, we have characterized the molecular mechanisms behind a gain-of-function phenotype of Tap46 and its relationship with TOR to gain further insights into Tap46 function in plants. Constitutive overexpression of Tap46 in Arabidopsis resulted in overall growth stimulation with enlarged organs, such as leaves and siliques. Kinematic analysis of leaf growth revealed that increased cell size was mainly responsible for the leaf enlargement. Tap46 overexpression also enhanced seed size and viability under accelerated ageing conditions. Enhanced plant growth was also observed in dexamethasone (DEX)-inducible Tap46 overexpression Arabidopsis lines, accompanied by increased cellular activities of nitrate-assimilating enzymes. DEX-induced Tap46 overexpression and Tap46 RNAi resulted in increased and decreased phosphorylation of S6 kinase (S6K), respectively, which is a sensitive indicator of endogenous TOR activity, and Tap46 interacted with S6K in planta based on bimolecular fluorescence complementation and co-immunoprecipitation. Furthermore, inactivation of TOR by estradiol-inducible RNAi or rapamycin treatment decreased Tap46 protein levels, but increased PP2A catalytic subunit levels. Real-time quantitative PCR analysis revealed that Tap46 overexpression induced transcriptional modulation of genes involved in nitrogen metabolism, ribosome biogenesis, and lignin biosynthesis. These findings suggest that Tap46 modulates plant growth as a positive effector of the TOR signalling pathway and Tap46/PP2Ac protein abundance is regulated by TOR activity.

J. Exp. Bot. Feb. 01, 2015; 66(3);827-40 [PUBMED:25399018]

**31. Membrane-localized extra-large G proteins and Gbg of the heterotrimeric G proteins form functional complexes engaged in plant immunity in Arabidopsis.**Maruta N, Trusov Y, Brenya E, Parekh U, Botella JR

In animals, heterotrimeric G proteins, comprising Ga, Gb, and Gg subunits, are molecular switches whose function tightly depends on Ga and Gbg interaction. Intriguingly, in Arabidopsis (Arabidopsis thaliana), multiple defense responses involve Gbg, but not Ga. We report here that the Gbg dimer directly partners with extra-large G proteins (XLGs) to mediate plant immunity. Arabidopsis mutants deficient in XLGs, Gb, and Gg are similarly compromised in several pathogen defense responses, including disease development and production of reactive oxygen species. Genetic analysis of double, triple, and quadruple mutants confirmed that XLGs and Gbg functionally interact in the same defense signaling pathways. In addition, mutations in XLG2 suppressed the seedling lethal and cell death phenotypes of BRASSINOSTEROID INSENSITIVE1-associated receptor kinase1-interacting receptor-like kinase1 mutants in an identical way as reported for Arabidopsis Gb-deficient mutants. Yeast (Saccharomyces cerevisiae) three-hybrid and bimolecular fluorescent complementation assays revealed that XLG2 physically interacts with all three possible Gbg dimers at the plasma membrane. Phylogenetic analysis indicated a close relationship between XLGs and plant Ga subunits, placing the divergence point at the dawn of land plant evolution. Based on these findings, we conclude that XLGs form functional complexes with Gbg dimers, although the mechanism of action of these complexes, including activation/deactivation, must be radically different form the one used by the canonical Ga subunit and are not likely to share the same receptors. Accordingly, XLGs expand the repertoire of heterotrimeric G proteins in plants and reveal a higher level of diversity in heterotrimeric G protein signaling.

Plant Physiol. Mar. 01, 2015; 167(3);1004-16 [PUBMED:25588736]

**32. Methyl-CpG-binding domain protein MBD7 is required for active DNA demethylation in Arabidopsis.**Wang C, Dong X, Jin D, Zhao Y, Xie S, Li X, He X, Lang Z, Lai J, Zhu JK, Gong Z

Although researchers have established that DNA methylation and active demethylation are dynamically regulated in plant cells, the molecular mechanism for the regulation of active DNA demethylation is not well understood. By using an Arabidopsis (Arabidopsis thaliana) line expressing the Promoter RESPONSIVE TO DEHYDRATION 29A:LUCIFERASE (ProRD29A:LUC) and Promoter cauliflower mosaic virus 35S:NEOMYCIN PHOSPHOTRANSFERASE II (Pro35S:NPTII) transgenes, we isolated an mbd7 (for methyl-CpG-binding domain protein7) mutant. The mbd7 mutation causes an inactivation of the Pro35S:NPTII transgene but does not affect the expression of the ProRD29A:LUC transgene. The silencing of the Pro35S:NPTII reporter gene is associated with DNA hypermethylation of the reporter gene. MBD7 interacts physically with REPRESSOR OF SILENCING5/INCREASED DNA METHYLATION2, a protein in the small heat shock protein family. MBD7 prefers to target the genomic loci with high densities of DNA methylation around chromocenters. The Gypsy-type long terminal repeat retrotransposons mainly distributed around chromocenters are most affected by mbd7 in all transposons. Our results suggest that MBD7 is required for active DNA demethylation and antisilencing of the genomic loci with high densities of DNA methylation in Arabidopsis.

Plant Physiol. Mar. 01, 2015; 167(3);905-14 [PUBMED:25593350]

**33. Destabilization of interaction between cytokinin signaling intermediates AHP1 and ARR4 modulates Arabidopsis development.**Verma V, Sivaraman J, Srivastava AK, Sadanandom A, Kumar PP

Eukaryotic two-component signaling involves the His-Asp-His-Asp multistep phosphorelay (MSP). In Arabidopsis thaliana, cytokinin-mediated MSP signaling intermediates include histidine kinases (HKs), histidine phosphotransfer proteins (Hpts) and response regulators (RRs). The structure-function relationship of interaction between Hpt (e.g. AHP1) and RR (e.g. ARR4) is poorly understood. Using a homology model and yeast two-hybrid analysis, we identified key amino acids of ARR4 at the AHP1-ΔARR4((16-175)) interaction interface. Mutating them in Arabidopsis (arr3,4,5,6,8,9 hextuple mutant background) and performing root length assays provided functional relevance, and coimmunoprecipitation (coIP) assay provided biochemical evidence for the interaction. The homology model mimics crystal structures of Hpt-RR complexes. Mutating selected interface residues of ARR4 either abolished or destabilized the interaction. D45A and Y96A mutations weakened interaction with AHP1, and exhibited weaker rescue of root elongation in the hextuple mutants. CoIP analysis using cytokinin-treated transgenic Arabidopsis seedlings provided biochemical evidence for weakened AHP1-ARR4 interaction. The relevance of the selected residues for the interaction was further validated in two independent pairs of Hpt-RR proteins from Arabidopsis and rice (Oryza sativa). Our data provide evidence of a link between Hpt-RR interaction affinity and regulation of downstream functions of RRs. This establishes a structure-function relationship for the final step of a eukaryotic MSP signal cascade.

New Phytol. Apr. 01, 2015; 206(2);726-37 [PUBMED:25643735]

**34. The Arabidopsis thaliana RNA-binding protein FCA regulates thermotolerance by modulating the detoxification of reactive oxygen species.**Lee S, Lee HJ, Jung JH, Park CM

Heat stress affects various aspects of plant growth and development by generating reactive oxygen species (ROS) which cause oxidative damage to cellular components. However, the mechanisms by which plants cope with ROS accumulation during their thermotolerance response remain largely unknown. Here, we demonstrate that the RNA-binding protein FCA, a key component of flowering pathways in Arabidopsis thaliana, is required for the acquisition of thermotolerance. Transgenic plants overexpressing the FCA gene (35S:FCA) were resistant to heat stress; the FCA-defective fca-9 mutant was sensitive to heat stress, consistent with induction of the FCA gene by heat. Furthermore, total antioxidant capacity was higher in the 35S:FCA transgenic plants but lower in the fca-9 mutant compared with wild-type controls. FCA interacts with the ABA-INSENSITIVE 5 (ABI5) transcription factor, which regulates the expression of genes encoding antioxidants, including 1-CYSTEINE PEROXIREDOXIN 1 (PER1). We found that FCA is needed for proper expression of the PER1 gene by ABI5. Our observations indicate that FCA plays a role in the induction of thermotolerance by triggering antioxidant accumulation under heat stress conditions, thus providing a novel role for FCA in heat stress responses in plants.

New Phytol. Jan. 01, 2015; 205(2);555-69 [PUBMED:25266977]

**35. An abscisic acid inducible Arabidopsis MAPKKK, MAPKKK18 regulates leaf senescence via its kinase activity.**Matsuoka D, Yasufuku T, Furuya T, Nanmori T

Abscisic acid (ABA) is a phytohormone that regulates many physiological functions, such as plant growth, development and stress responses. The MAPK cascade plays an important role in ABA signal transduction. Several MAPK and MAPKK molecules are reported to function in ABA signaling; however, there have been few studies related to the identification of MAPKKK upstream of MAPKK in ABA signaling. In this study, we show that an Arabidopsis MAPKKK, MAPKKK18 functions in ABA signaling. The expression of MAPKKK18 was induced by ABA treatment. Yeast two-hybrid analysis revealed that MAPKKKK18 interacted with MKK3, which interacted with C-group MAPK, MPK1/2/7. Immunoprecipitated kinase assay showed that the 3xFlag-tagged MAPKKK18, expressed in Arabidopsis plants, was activated when treated with ABA. These results indicate the possibility that the MAPK cascade is composed of MAPKKK18, MKK3 and MPK1/2/7 in ABA signaling. The transgenic plants overexpressing MAPKKK18 (35S:MAPKKK18) and its kinase negative mutant (35S:MAPKKK18 KN) were generated, and their growth was monitored. Compared with the WT plant, 35S:MAPKKK18 and 35S:MAPKKK18 KN showed smaller and bigger phenotypes, respectively. Senescence of the rosette leaves was promoted in 35S:MAPKKK18, but suppressed in 35S:MAPKKK18 KN. Furthermore, ABA-induced leaf senescence was accelerated in 35S:MAPKKK18. These results suggest that MAPKKK18 controls the plant growth by adjusting the timing of senescence via its protein kinase activity in ABA dependent manners.

Mesh Terms:

Abscisic Acid, Amino Acid Sequence, Arabidopsis, Arabidopsis Proteins, Gene Expression, Gene Expression Regulation, Plant, MAP Kinase Kinase Kinases, Phosphorylation, Plant Growth Regulators, Plant Leaves, Plants, Genetically Modified, Recombinant Proteins, Seedling, Seeds, Signal Transduction, Stress, Physiological, Time Factors, Two-Hybrid System Techniques, Water

Plant Mol. Biol. Apr. 01, 2015; 87(6);565-75 [PUBMED:25680457]

**36. GIP1 protein is a novel cofactor that regulates DNA-binding affinity of redox-regulated members of bZIP transcription factors involved in the early stages of Arabidopsis development.**Shaikhali J

In response to environmental light signals, gene expression adjustments play an important role in regulation of photomorphogenesis. LHCB2.4 is among the genes responsive to light signals, and its expression is regulated by redox-regulated members of G-group bZIP transcription factors. The biochemical interrelations of GBF1-interacting protein 1 (GIP1) and the G-group bZIP transcription factors have been investigated. GIP1, previously shown to enhance DNA-binding activities of maize GBF1 and Arabidopsis GBF3, is a plant specific protein that reduces DNA-binding activity of AtbZIP16, AtbZIP68, and AtGBF1 under non-reducing conditions through direct physical interaction shown by the yeast two-hybrid and pull-down assays. Fluorescence microscopy studies using cyan fluorescent protein (CFP)-fusion protein indicate that GIP1 is exclusively localized in the nucleus. Under non- reducing conditions, GIP1 exhibits predominantly high molecular weight forms, whereas it predominates in low molecular weight monomers under reducing conditions. While reduced GIP1 induced formation of DNA-protein complexes of G-group bZIPs, oxidized GIP1 decreased the amount of those complexes and instead induced its chaperone function suggesting functional switching from redox to chaperone activity. Finally analysis of transgenic plants overexpressing GIP1 revealed that GIP1 is a negative co-regulator in red and blue light mediated hypocotyl elongation. By regulating the repression effect by bZIP16 and the activation effect by bZIP68 and GBF1 on LHCB2.4 expression, GIP1 functions to promote hypocotyl elongation during the early stages of Arabidopsis seedling development.

Protoplasma May. 01, 2015; 252(3);867-83 [PUBMED:25387999]

View in: Pubmed|Google Scholar

**37. Structural basis for recognition of an endogenous peptide by the plant receptor kinase PEPR1.**Tang J, Han Z, Sun Y, Zhang H, Gong X, Chai J

The endogenous peptides AtPep1-8 in Arabidopsis mature from the conserved C-terminal portions of their precursor proteins PROPEP1-8, respectively. The two homologous leucine-rich repeat-receptor kinases (LRR-RKs) PEPR1 and PEPR2 act as receptors of AtPeps. AtPep binding leads to stable association of PEPR1,2 with the shared receptor LRR-RK BAK1, eliciting immune responses similar to those induced by pathogens. Here we report a crystal structure of the extracellular LRR domain of PEPR1 (PEPR1LRR) in complex with AtPep1. The structure reveals that AtPep1 adopts a fully extended conformation and binds to the inner surface of the superhelical PEPR1LRR. Biochemical assays showed that AtPep1 is capable of inducing PEPR1LRR-BAK1LRR heterodimerization. The conserved C-terminal portion of AtPep1 dominates AtPep1 binding to PEPR1LRR, with the last amino acid of AtPep1 Asn23 forming extensive interactions with PEPR1LRR. Deletion of the last residue of AtPep1 significantly compromised AtPep1 interaction with PEPR1LRR. Together, our data reveal a conserved structural mechanism of AtPep1 recognition by PEPR1, providing significant insight into prediction of recognition of other peptides by their cognate LRR-RKs.

Cell Res. Jan. 01, 2015; 25(1);110-20 [PUBMED:25475059]

**38. A zinc finger motif-containing protein is essential for chloroplast RNA editing.**Sun T, Shi X, Friso G, Van Wijk K, Bentolila S, Hanson MR

C-to-U editing of transcripts in plant organelles is carried out by small (<400 kD) protein complexes called editosomes. Recognition of the proper C target for editing is mediated by pentatricopeptide repeat (PPR) containing proteins that recognize cis-elements. Members of two additional gene families, the RIP/MORF and ORRM families, have each been found to be required for editing of particular sets of Cs in mitochondria and/or chloroplasts. By co-immunoprecipitation of the chloroplast editing factor ORRM1, followed by mass spectrometry, we have now identified a member of the RanBP2 type zinc fingers (pFAM00641) protein family that is required for editing of 14 sites in chloroplasts and affects editing efficiency of another 16 chloroplast C targets. In yeast two-hybrid assays, OZ1 (Organelle Zinc finger 1) interacts with PPR site recognition factors whose cognate sites are affected when OZ1 is mutated. No interaction of OZ1 with the chloroplast editing factors RIP2 and RIP9 was detected; however, OZ1 interacts with ORRM1, which binds to RIP proteins, allowing us to build a model for the chloroplast RNA editosome. The RNA editosomes that act upon most chloroplast C targets are likely to contain a PPR protein recognition factor, either RIP2 or RIP9, ORRM1, and OZ1. The organelle zinc finger editing factor family (OZ) contains 4 members in Arabidopsis, three that are predicted to be targeted to chloroplasts and one to mitochondria. With the identification of OZ1, there are now 4 nuclear-encoded protein families known to be essential for plant organelle RNA editing.

PLoS Genet. Mar. 01, 2015; 11(3);e1005028 [PUBMED:25768119]

**39. FYVE1 is essential for vacuole biogenesis and intracellular trafficking in Arabidopsis.**Kolb C, Nagel MK, Kalinowska K, Hagmann J, Ichikawa M, Anzenberger F, Alkofer A, Sato MH, Braun P, Isono E

The plant vacuole is a central organelle that is involved in various biological processes throughout the plant life cycle. Elucidating the mechanism of vacuole biogenesis and maintenance is thus the basis for our understanding of these processes. Proper formation of the vacuole has been shown to depend on the intracellular membrane trafficking pathway. Although several mutants with altered vacuole morphology have been characterized in the past, the molecular basis for plant vacuole biogenesis has yet to be fully elucidated. With the aim to identify key factors that are essential for vacuole biogenesis, we performed a forward genetics screen in Arabidopsis (Arabidopsis thaliana) and isolated mutants with altered vacuole morphology. The vacuolar fusion defective1 (vfd1) mutant shows seedling lethality and defects in central vacuole formation. VFD1 encodes a Fab1, YOTB, Vac1, and EEA1 (FYVE) domain-containing protein, FYVE1, that has been implicated in intracellular trafficking. FYVE1 localizes on late endosomes and interacts with Src homology-3 domain-containing proteins. Mutants of FYVE1 are defective in ubiquitin-mediated protein degradation, vacuolar transport, and autophagy. Altogether, our results show that FYVE1 is essential for plant growth and development and place FYVE1 as a key regulator of intracellular trafficking and vacuole biogenesis.

Plant Physiol. Apr. 01, 2015; 167(4);1361-73 [PUBMED:25699591]

**40. An AP endonuclease functions in active DNA dimethylation and gene imprinting in arabidopsis.**Li Y, Cordoba-Canero D, Qian W, Zhu X, Tang K, Zhang H, Ariza RR, Roldan-Arjona T, Zhu JK

Active DNA demethylation in plants occurs through base excision repair, beginning with removal of methylated cytosine by the ROS1/DME subfamily of 5-methylcytosine DNA glycosylases. Active DNA demethylation in animals requires the DNA glycosylase TDG or MBD4, which functions after oxidation or deamination of 5-methylcytosine, respectively. However, little is known about the steps following DNA glycosylase action in the active DNA demethylation pathways in plants and animals. We show here that the Arabidopsis APE1L protein has apurinic/apyrimidinic endonuclease activities and functions downstream of ROS1 and DME. APE1L and ROS1 interact in vitro and co-localize in vivo. Whole genome bisulfite sequencing of ape1l mutant plants revealed widespread alterations in DNA methylation. We show that the ape1l/zdp double mutant displays embryonic lethality. Notably, the ape1l+/-zdp-/- mutant shows a maternal-effect lethality phenotype. APE1L and the DNA phosphatase ZDP are required for FWA and MEA gene imprinting in the endosperm and are important for seed development. Thus, APE1L is a new component of the active DNA demethylation pathway and, together with ZDP, regulates gene imprinting in Arabidopsis.

PLoS Genet. Jan. 01, 2015; 11(1);e1004905 [PUBMED:25569774]

**41. Evolution of the Telomere-Associated Protein POT1a in Arabidopsis thaliana Is Characterized by Positive Selection to Reinforce Protein-Protein Interaction.**Beilstein MA, Renfrew KB, Song X, Shakirov EV, Zanis MJ, Shippen DE

Gene duplication is a major driving force in genome evolution. Here, we explore the nature and origin of the POT1 gene duplication in Arabidopsis thaliana. Protection of Telomeres (POT1) is a conserved multifunctional protein that modulates telomerase activity and its engagement with telomeres. Arabidopsis thaliana encodes two divergent POT1 paralogs termed AtPOT1a and AtPOT1b. AtPOT1a positively regulates telomerase activity, whereas AtPOT1b is proposed to negatively regulate telomerase and promote chromosome end protection. Phylogenetic analysis uncovered two independent POT1 duplication events in the plant kingdom, including one at the base of Brassicaceae. Tests for positive selection implemented in PAML revealed that the Brassicaceae POT1a lineage experienced positive selection postduplication and identified three amino acid residues with signatures of positive selection. A sensitive and quantitative genetic complementation assay was developed to assess POT1a function in A. thaliana. The assay showed that AtPOT1a is functionally distinct from single-copy POT1 genes in other plants. Moreover, for two of the sites with a strong signature of positive selection, substitutions that swap the amino acids in AtPOT1a for residues found in AtPOT1b dramatically compromised AtPOT1a function in vivo. In vitro-binding studies demonstrated that all three sites under positive selection specifically enhance the AtPOT1a interaction with CTC1, a core component of the highly conserved CST (CTC1/STN1/TEN1) telomere protein complex. Our results reveal a molecular mechanism for the role of these positively selected sites in AtPOT1a. The data also provide an important empirical example to refine theories of duplicate gene retention, as the outcome of positive selection here appears to be reinforcement of an ancestral function, rather than neofunctionalization. We propose that this outcome may not be unusual when the duplicated protein is a component of a multisubunit complex whose function is in part specified by other members.

Mol. Biol. Evol. May. 01, 2015; 32(5);1329-41 [PUBMED:25697340]

**42. The glucosinolate breakdown product indole-3-carbinol acts as an auxin antagonist in roots of Arabidopsis thaliana.**

Katz E, Nisani S, Yadav BS, Woldemariam MG, Shai B, Obolski U, Ehrlich M, Shani E, Jander G, Chamovitz DA

The glucosinolate breakdown product indole-3-carbinol functions in cruciferous vegetables as a protective agent against foraging insects. While the toxic and deterrent effects of glucosinolate breakdown on herbivores and pathogens have been studied extensively, the secondary responses that are induced in the plant by indole-3-carbinol remain relatively uninvestigated. Here we examined the hypothesis that indole-3-carbinol plays a role in influencing plant growth and development by manipulating auxin signaling. We show that indole-3-carbinol rapidly and reversibly inhibits root elongation in a dose-dependent manner, and that this inhibition is accompanied by a loss of auxin activity in the root meristem. A direct interaction between indole-3-carbinol and the auxin perception machinery was suggested, as application of indole-3-carbinol rescues auxin-induced root phenotypes. In vitro and yeast-based protein interaction studies showed that indole-3-carbinol perturbs the auxin-dependent interaction of Transport Inhibitor Response (TIR1) with auxin/3-indoleacetic acid (Aux/IAAs) proteins, further supporting the possibility that indole-3-carbinol acts as an auxin antagonist. The results indicate that chemicals whose production is induced by herbivory, such as indole-3-carbinol, function not only to repel herbivores, but also as signaling molecules that directly compete with auxin to fine tune plant growth and development.

Plant J. May. 01, 2015; 82(4);547-55 [PUBMED:25758811]

**43. AtLa1 protein initiates IRES-dependent translation of WUSCHEL mRNA and regulates the stem cell homeostasis of Arabidopsis in response to environmental hazards.**Cui Y, Rao S, Chang B, Wang X, Zhang K, Hou X, Zhu X, Wu H, Tian Z, Zhao Z, Yang C, Huang T

Plant stem cells are hypersensitive to environmental hazards throughout their life cycle, but the mechanism by which plants safeguard stem cell homeostasis in response to environmental hazards is largely unknown. The homeodomain transcription factor WUSCHEL (WUS) protein maintains the stem cell pool in the shoot apical meristem of Arabidopsis. Here, we demonstrate that the translation of WUS mRNA is directed by an internal ribosomal entry site (IRES) located in the 5'-untranslated region. The AtLa1 protein, an RNA-binding factor, binds to the 5'-untranslated region and initiates the IRES-dependent translation of WUS mRNA. Knockdown of AtLa1 expression represses the WUS IRES-dependent translation and leads to the arrest of growth and development. The AtLa1 protein is mainly located in the nucleoplasm. However, environmental hazards promote the nuclear-to-cytoplasmic translocation of the AtLa1 protein, which further enhances the IRES-dependent translation of WUS mRNA. Genetic evidence indicates that the WUS protein increases the tolerance of the shoot apical meristem to environmental hazards. Based on these results, we conclude that the stem cell niche in Arabidopsis copes with environmental hazards by enhancing the IRES-dependent translation of WUS mRNA under the control of the AtLa1 protein.

Plant Cell Environ. Mar. 12, 2015; 0(0); [PUBMED:25764476]

**44. The Arabidopsis transcription factor BRASSINOSTEROID INSENSITIVE1-ETHYL METHANESULFONATE-SUPPRESSOR1 is a direct substrate of MITOGEN-ACTIVATED PROTEIN KINASE6 and regulates immunity.**Kang S, Yang F, Li L, Chen H, Chen S, Zhang J

Pathogen-associated molecular patterns (PAMPs) are recognized by plant pattern recognition receptors to activate PAMP-triggered immunity (PTI). Mitogen-activated protein kinases (MAPKs), as well as other cytoplasmic kinases, integrate upstream immune signals and, in turn, dissect PTI signaling via different substrates to regulate defense responses. However, only a few direct substrates of these signaling kinases have been identified. Here, we show that PAMP perception enhances phosphorylation of BRASSINOSTEROID INSENSITIVE1-ETHYL METHANESULFONATE-SUPPRESSOR1 (BES1), a transcription factor involved in brassinosteroid (BR) signaling pathway, through pathogen-induced MAPKs in Arabidopsis (Arabidopsis thaliana). BES1 interacts with MITOGEN-ACTIVATED PROTEIN KINASE6 (MPK6) and is phosphorylated by MPK6. bes1 loss-of-function mutants display compromised resistance to bacterial pathogen Pseudomonas syringae pv tomato DC3000. BES1 S286A/S137A double mutation (BES1(SSAA)) impairs PAMP-induced phosphorylation and fails to restore bacterial resistance in bes1 mutant, indicating a positive role of BES1 phosphorylation in plant immunity. BES1 is phosphorylated by glycogen synthase kinase3 (GSK3)-like kinase BR-insensitive2 (BIN2), a negative regulator of BR signaling. BR perception inhibits BIN2 activity, allowing dephosphorylation of BES1 to regulate plant development. However, BES1(SSAA) does not affect BR-mediated plant growth, suggesting differential residue requirements for the modulation of BES1 phosphorylation in PTI and BR signaling. Our study identifies BES1 as a unique direct substrate of MPK6 in PTI signaling. This finding reveals MAPK-mediated BES1 phosphorylation as another BES1 modulation mechanism in plant cell signaling, in addition to GSK3-like kinase-mediated BES1 phosphorylation and F box protein-mediated BES1 degradation.Plant Physiol. Mar. 01, 2015; 167(3);1076-86 [PUBMED:25609555]

**45. Functional roles of the hexamer organization of plant glutamate decarboxylase.**Astegno A, Capitani G, Dominici P

Glutamate decarboxylase (GAD) is a pyridoxal 5'-phosphate (PLP)-dependent enzyme that catalyzes the α-decarboxylation of glutamate to γ-aminobutyrate. A unique feature of plant GAD is the presence of a calmodulin (CaM)-binding domain at its C-terminus. In plants, transient elevation of cytosolic Ca(2+) in response to different types of stress is responsible for GAD activation via CaM. The crystal structure of GAD isoform 1 from Arabidopsis thaliana (AtGAD1) shows that the enzyme is a hexamer composed of a trimer of dimers. Herein, we show that in solution AtGAD1 is in a dimer-hexamer equilibrium and estimate the dissociation constant (Kd) for the hexamer under different conditions. The association of dimers into hexamers is promoted by several conditions, including high protein concentrations and low pH. Notably, binding of Ca(2+)/CaM1 abolishes the dissociation of the AtGAD1 oligomer. The AtGAD1 N-terminal domain is critical for maintaining the oligomeric state as removal of the first 24 N-terminal residues dramatically affects oligomerization by producing a dimeric enzyme. The deleted mutant retains decarboxylase activity, highlighting the dimeric nature of the basic structural unit of AtGAD1. Site-directed mutagenesis identified Arg24 in the N-terminal domain as a key residue since its mutation to Ala prevents hexamer formation in solution. Both dimeric mutant enzymes form a stable hexamer in the presence of Ca(2+)/CaM1. Our data clearly reveal that the oligomeric state of AtGAD1 is highly responsive to a number of experimental parameters and may have functional relevance in vivo in the light of the biphasic regulation of AtGAD1 activity by pH and Ca(2+)/CaM1 in plant cells. This article is part of a special issue titled "Cofactor-Dependent Proteins: Evolution, Chemical Diversity and Bio-applications."

Biochim. Biophys. Acta Jan. 19, 2015; 0(0); [PUBMED:25614413]

**46. A high molecular mass zinc transporter MTP12 forms a functional heteromeric complex with MTP5 in the Golgi in Arabidopsis thaliana.**

Fujiwara T, Kawachi M, Sato Y, Mori H, Kutsuna N, Hasezawa S, Maeshima M

Zinc (Zn) is an essential micronutrient required for plant growth and development. In Arabidopsis thaliana, several families of Zn transporters engaged in Zn import, export and intracellular compartmentalization play important roles in Zn homeostasis. We describe a novel Zn transporter, A. thaliana metal tolerance protein 12 (AtMTP12), which belongs to the cation diffusion facilitator family. AtMTP12 is predicted to consist of 798 amino acids and have 14 transmembrane segments. The expression of AtMTP12 in suspension-cultured cells was not affected by Zn deficiency or excess. Heterologous expression in a mutant of budding yeast (Saccharomyces cerevisiae) that lacks Msc2p, an orthologue of AtMTP12, revealed that AtMTP12 complements the growth phenotype of the msc2 mutant when AtMTP5t1, one of the splicing variants of AtMTP5, is coexpressed. Transient expression of AtMTP12-fused green fluorescent protein in A. thaliana mesophyll protoplasts demonstrated that AtMTP12 is localized to the Golgi apparatus. Moreover, AtMTP12 and AtMTP5t1 interact in the Golgi, as determined by a bimolecular fluorescence complementation assay. These results suggest that AtMTP12 forms a functional complex with AtMTP5t1 to transport Zn into the Golgi.Nucleotide sequence data for full-length of AtMTP12 is available in the DDBJ/EMBL/GenBank database under accession number AB986563.

FEBS J. Mar. 03, 2015; 0(0); [PUBMED:25732056]

**47. Diffuse decapping enzyme DCP2 accumulates in DCP1 foci under heat stress in Arabidopsis thaliana.**Motomura K, Le QT, Hamada T, Kutsuna N, Mano S, Nishimura M, Watanabe Y

The decapping enzymes DCP1 and DCP2 are components of a decapping coProtein-protein interaction and gene co-expression maps of ARFs and Aux/IAAs in Arabidopsis.

Piya S, Shrestha SK, Binder B, Stewart CN, Hewezi T

The phytohormone auxin regulates nearly all aspects of plant growth and development. Based on the current model in Arabidopsis thaliana, Auxin/indole-3-acetic acid (Aux/IAA) proteins repress auxin-inducible genes by inhibiting auxin response transcription factors (ARFs). Experimental evidence suggests that heterodimerization between Aux/IAA and ARF proteins are related to their unique biological functions. The objective of this study was to generate the Aux/IAA-ARF protein-protein interaction map using full length sequences and locate the interacting protein pairs to specific gene co-expression networks in order to define tissue-specific responses of the Aux/IAA-ARF interactome. Pairwise interactions between 19 ARFs and 29 Aux/IAAs resulted in the identification of 213 specific interactions of which 79 interactions were previously unknown. The incorporation of co-expression profiles with protein-protein interaction data revealed a strong correlation of gene co-expression for 70% of the ARF-Aux/IAA interacting pairs in at least one tissue/organ, indicative of the biological significance of these interactions. Importantly, ARF4-8 and 19, which were found to interact with almost all Aux-Aux/IAA showed broad co-expression relationships with Aux/IAA genes, thus, formed the central hubs of the co-expression network. Our analyses provide new insights into the biological significance of ARF-Aux/IAA associations in the morphogenesis and development of various plant tissues and organs.

Front Plant Sci Jan. 08, 2015; 5(0);744 [PUBMED:25566309]

**48. Structural insight into the conformational change of alcohol dehydrogenase from Arabidopsis thaliana L. during coenzyme binding.**

Chen F, Wang P, An Y, Huang J, Xu Y

Alcohol dehydrogenase (ADH, EC 1.1.1.1) plays important roles in the metabolism of alcohols and aldehydes. They are often subjected to conformational changes that are critical for the enzymatic activity and have received intensive investigation for horse liver ADH. However, for the large plant ADH members, little is known regarding both the conformational change and its relationship to catalytic activity as plant ADH structures were rarely available. Here we describe three Arabidopsis ADH conformations obtained from two crystals, the apo crystal that was free of ligand, and the complex crystal that was with NAD. The NAD-complexed crystal yielded two different structural forms for the two subunits, one was occupied by the coenzyme, and the other was free and open. Structural comparisons demonstrate that the occupied subunit is in a closed conformation while the free subunit is fully open, and the apo structure in intermediate. Though all the forms have an overall fold similar to that of horse and human ADHs, the catalytic domain has an over 10° rotation. Additionally, unlike horse liver ADH, the loop (295-302aa) adopts different conformation. It does not rearrange upon the binding of the coenzyme norVal297 side chain experiences a flipping. Instead it always remains in the active site. His48 plays a switching role in the structure. Its imidazole ring has to swim away from the binding site to permit NAD binding. These together with the large differences in the substrate binding pocket, as well as in the proton relay system demonstrate that AtADH adopts a different catalysis mechanism from horse liver ADH.

Biochimie Jan. 01, 2015; 108(0);33-9 [PUBMED:25447145]

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**49. The PHD-finger module of the Arabidopsis thaliana defense regulator EDM2 can recognize triply modified histone H3 peptides.**Tsuchiya T, Eulgem T

Recently we reported that the Arabidopsis thaliana PHD-finger protein EDM2 (enhanced downy mildew 2) impacts disease resistance by affecting levels of di-methylated lysine 9 of histone H3 (H3K9me2) at an alternative polyadenylation site in the immune receptor gene RPP7. EDM2-dependent modulation of this post-translational histone modification (PHM) shifts the balance between full-length RPP7 transcripts and prematurely polyadenylated transcripts, which do not encode the RPP7 protein. Our previous work genetically linked, for the first time, PHMs to alternative polyadenylation and established EDM2 as a critical component mediating PHM-dependent polyadenylation control. However, how EDM2 is recruited to its genomic target sites and how it affects H3K9me2 levels is unknown. Here we show the PHD-finger module of EDM2 to recognize histone H3 bearing certain combinations of 3 distinct PHMs. Our results suggest that targeting of EDM2 to specific genomic regions is mediated by the histone-binding selectivity of its PHD-finger domain.

Plant Signal Behav Mar. 13, 2015; 9(7);e29202 [PUBMED:25763495]

**50. Two activities of long-chain acyl-coenzyme A synthetase are involved in lipid trafficking between the endoplasmic reticulum and the plastid in Arabidopsis.**Jessen D, Roth C, Wiermer M, Fulda M

In plants, fatty acids are synthesized within the plastid and need to be distributed to the different sites of lipid biosynthesis within the cell. Free fatty acids released from the plastid need to be converted to their corresponding coenzyme A thioesters to become metabolically available. This activation is mediated by long-chain acyl-coenzyme A synthetases (LACSs), which are encoded by a family of nine genes in Arabidopsis (Arabidopsis thaliana). So far, it has remained unclear which of the individual LACS activities are involved in making plastid-derived fatty acids available to cytoplasmic glycerolipid biosynthesis. Because of its unique localization at the outer envelope of plastids, LACS9 was regarded as a candidate for linking plastidial fatty export and cytoplasmic use. However, data presented in this study show that LACS9 is involved in fatty acid import into the plastid. The analyses of mutant lines revealed strongly overlapping functions of LACS4 and LACS9 in lipid trafficking from the endoplasmic reticulum to the plastid. In vivo labeling experiments with lacs4 lacs9 double mutants suggest strongly reduced synthesis of endoplasmic reticulum-derived lipid precursors, which are required for the biosynthesis of glycolipids in the plastids. In conjunction with this defect, double-mutant plants accumulate significant amounts of linoleic acid in leaf tissue.

Plant Physiol. Feb. 01, 2015; 167(2);351-66 [PUBMED:25540329]