Lit Lunch 3-27-13

Role and Interrelationship of GaProtein, Hydrogen Peroxide, and Nitric Oxide in Ultraviolet B-Induced Stomatal Closure in Arabidopsis Leaves

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Heterotrimeric G proteins have been shown to transmit ultraviolet B (UV-B) signals in mammalian cells, but whether they alsotransmit UV-B signals in plant cells is not clear. In this paper, we report that 0.5 Wm22 UV-B induces stomatal closure inArabidopsis(Arabidopsis thaliana) by eliciting a cascade of intracellular signaling events including Gaprotein, hydrogen peroxide (H2O2), and nitric oxide (NO). UV-B triggered a significant increase in H2O2 or NO levels associated with stomatal closure in the wild type, but these effects were abolished in the single and double mutants of AtrbohDandAtrbohFor in theNia1mutants, respectively. Furthermore, we found that UV-B-mediated H2O2 and NO generation are regulated by GPA1, the Ga-subunit of heterotrimeric G proteins. UV-B-dependent H2O2 and NO accumulation were nullified ingpa1knockout mutants but enhanced by overexpression of a constitutively active form ofGPA1(cGa). In addition, exogenously applied H2O2or NO rescued the defect in UV-B-mediated stomatal closure ingpa1mutants, whereas cGaAtrbohD/AtrbohFand cGania1constructs exhibited a similar response toAtrbohD/AtrbohF and Nia1, respectively. Finally, we demonstrated that Gaactivation of NO production depends on H2O2. The mutants of AtrbohDandAtrbohFhad impaired NO generation in response to UV-B, but UV-B-induced H2O2accumulation was not impaired in Nia1. Moreover, exogenously applied NO rescued the defect in UV-B-mediated stomatal closure in the mutants of AtrbohDand AtrbohF.Thesefindings establish a signaling pathway leading to UV-B-induced stomatal closure that involves GPA1-dependent activation of H2O2 production and subsequent Nia1-dependent NO accumulation.

SlARF4, an Auxin Response Factor Involved in the Control of Sugar Metabolism during Tomato Fruit Development

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Successful completion of fruit developmental programs depends on the interplay between multiple phytohormones. However, besides ethylene, the impact of other hormones on fruit quality traits remains elusive. A previous study has shown that down-regulation of SlARF4, a member of the tomato (Solanum lycopersicum) auxin response factor (ARF) gene family, results in a dark-green fruit phenotype with increased chloroplasts (Jones et al., 2002). This study further examines the role of this auxin transcriptional regulator during tomato fruit development at the level of transcripts, enzyme activities, and metabolites. It is noteworthy that the dark-green phenotype of antisense SlARF4-suppressed lines is restricted to fruit, suggesting that SlARF4 controls chlorophyll accumulation specifically in this organ. TheSlARF4 underexpressing lines accumulate more starch at early stages of fruit development and display enhanced chlorophyll content and photochemical efficiency, which is consistent with the idea that fruit photosynthetic activity accounts for the elevated starch levels. SlARF4expression is high in pericarp tissues of immature fruit and then undergoes a dramatic decline at the onset of ripening concomitant with the increase in sugar content. The higher starch content in developing fruits ofSlARF4down-regulated lines correlates with the up-regulation of genes and enzyme activities involved in starch biosynthesis, suggesting their negative regulation by SlARF4. Altogether, the data uncover the involvement of ARFs in the control of sugar content, an essential feature of fruit quality, and provide insight into the link between auxin signaling, chloroplastic activity, and sugar metabolism in developing fruit.

SAUR36,a SMALL AUXIN UP RNA Gene, Is Involved in the Promotion of Leaf Senescence in Arabidopsis

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Small Auxin Up RNA genes (SAURs) are early auxin-responsive genes, but whether any of them are involved in leaf senescence is not known. Auxin, on the other hand, has been shown to have a role in leaf senescence. Some of the external application experiments indicated that auxin can inhibit leaf senescence, whereas other experiments indicated that auxin can promote leaf senescence. Here, we report the identification and characterization of an Arabidopsis (Arabidopsis thaliana) leaf senescence-associated gene named SAG201, which is highly up-regulated during leaf senescence and can be induced by 1-naphthaleneacetic acid, a synthetic auxin. It encodes a functionally uncharacterized SAURthat has been annotated as SAUR36. Leaf senescence in transfer DNA insertion saur36 knockout lines was delayed as revealed by analyses of chlorophyll content,Fv/Fmratio (a parameter for photosystem II activity), ion leakage, and the expression of leaf senescence marker genes. In contrast, transgenic Arabidopsis plants overexpressingSAUR36(without its 39 untranslated region [UTR]) displayed an early leaf senescence phenotype. However, plants overexpressingSAUR36with its 39UTR were normal and did not exhibit the early-senescence phenotype. These data suggest that SAUR36 is a positive regulator of leaf senescence

and may mediate auxin-induced leaf senescence and that the 39UTR containing a highly conserved downstream destabilizes theSAUR36 transcripts in young leaves.

1. Science. 2013 Mar 1;339(6123):1067-70. doi: 10.1126/science.1230082.

KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants.

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Unlike animals, land plants undergo an alternation of generations, producing multicellular bodies in both haploid (1n: gametophyte) and diploid (2n: sporophyte) generations. Plant body plans in each generation are regulated by distinct developmental programs initiated at either meiosis or fertilization, respectively. In mosses, the haploid gametophyte generation is dominant, whereas in vascular plants-including ferns, gymnosperms, and angiosperms-the diploid sporophyte generation is dominant. Deletion of the class 2 KNOTTED1-LIKE HOMEOBOX (KNOX2) transcription factors in the moss Physcomitrella patens results in the development of gametophyte bodies from diploid embryos without meiosis. Thus, KNOX2 acts to prevent the haploid-specific body plan from developing in the diploid plant body, indicating a critical role for the evolution of KNOX2 in establishing an alternation of generations in land plants.

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2. Science. 2013 Mar 1;339(6123):1080-3. doi: 10.1126/science.1233066. Epub 2013 Feb 7.

Unraveling the mechanism of protein disaggregation through a ClpB-DnaK interaction.

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HSP-100 protein machines, such as ClpB, play an essential role in reactivating protein aggregates that can otherwise be lethal to cells. Although the players involved are known, including the DnaK/DnaJ/GrpE chaperone system in bacteria, details of the molecular interactions are not well understood. Using methyl-transverse relaxation-optimized nuclear magnetic resonance spectroscopy, we present an atomic-resolution model for the ClpB-DnaK complex, which we verified by mutagenesis and functional assays. ClpB and GrpE compete for binding to the DnaK nucleotide binding domain, with GrpE binding inhibiting disaggregation. DnaK, in turn, plays a dual role in both disaggregation and subsequent refolding of polypeptide chains as they emerge from the aggregate. On the basis of a combined structural-biochemical analysis, we propose a model for the mechanism of protein aggregate reactivation by ClpB.

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3. Science. 2013 Mar 15;339(6125):1335-8. doi: 10.1126/science.1232927. Epub 2013

Feb 7.

The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS.

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Comment in

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Expansion of a GGGGCC hexanucleotide repeat upstream of the C9orf72 coding region is the most common cause of familial frontotemporal lobar degeneration and amyotrophic lateral sclerosis (FTLD/ALS), but the pathomechanisms involved are unknown. As in other FTLD/ALS variants, characteristic intracellular inclusions of misfolded proteins define C9orf72 pathology, but the core proteins of the majority of inclusions are still unknown. Here, we found that most of these characteristic inclusions contain poly-(Gly-Ala) and, to a lesser extent, poly-(Gly-Pro) and poly-(Gly-Arg) dipeptide-repeat proteins presumably generated by non-ATG-initiated translation from the expanded GGGGCC repeat in three reading frames. These findings directly link the FTLD/ALS-associated genetic mutation to the predominant pathology in patients with C9orf72 hexanucleotide expansion.

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Journal of Biological Chemistry

The Molecular Basis of Iron-induced Oligomerization of Frataxin and the Role of the Ferroxidation Reaction in Oligomerization

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The role of the mitochondrial protein frataxin in iron storage and detoxification, iron delivery to iron-sulfur cluster biosynthesis, heme biosynthesis, and aconitase repair has been extensively studied during the last decade. However, still no general consensus exists on the details of the mechanism of frataxin function and oligomerization. Here, using small-angle x-ray scattering and x-ray crystallography, we describe the solution structure of the oligomers formed during the iron-dependent assembly of yeast (Yfh1) and Escherichia coli (CyaY) frataxin. At an iron-to-protein ratio of 2, the initially monomeric Yfh1 is converted to a trimeric form in solution. The trimer in turn serves as the assembly unit for higher order oligomers induced at higher iron-to-protein ratios. The x-ray crystallographic structure obtained from iron-soaked crystals demonstrates that iron binds at the trimer-trimer interaction sites, presumably contributing to oligomer stabilization. For the ferroxidation-deficient D79A/D82A variant of Yfh1, iron-dependent oligomerization may still take place, although >50% of the protein is found in the monomeric state at the highest iron-to-protein ratio used. This demonstrates that the ferroxidation reaction controls frataxin assembly and presumably the iron chaperone function of frataxin and its interactions with target proteins. For E. coli CyaY, the assembly unit of higher order oligomers is a tetramer, which could be an effect of the much shorter N-terminal region of this protein. The results show that understanding of the mechanistic features of frataxin function requires detailed knowledge of the interplay between the ferroxidation reaction, iron-induced oligomerization, and the structure of oligomers formed during assembly.

Phosphatidic Acid-dependent Recruitment and Function of the Rac Activator DOCK1 during Dorsal Ruffle Formation

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Activation of receptor tyrosine kinases leads to the formation of two different types of plasma membrane structures: peripheral ruffles and dorsal ruffles. Although the formation of both ruffle types requires activation of the small GTPase Rac, the difference in kinetics suggests that a distinct regulatory mechanism operates for their ruffle formation. DOCK1 and DOCK5 are atypical Rac activators and are both expressed in mouse embryonic fibroblasts (MEFs). We found that although PDGF-induced Rac activation and peripheral ruffle formation were coordinately regulated by DOCK1 and DOCK5 in MEFs, DOCK1 deficiency alone impaired dorsal ruffle formation in MEFs. Unlike DOCK5, DOCK1 bound to phosphatidic acid (PA) through the C-terminal polybasic amino acid cluster and was localized to dorsal ruffles. When this interaction was blocked, PDGF-induced dorsal ruffle formation was severely impaired. In addition, we show that phospholipase D, an enzyme that catalyzes PA synthesis, is required for PDGF-induced dorsal, but not peripheral, ruffle formation. These results indicate that the phospholipase D-PA axis selectively controls dorsal ruffle formation by regulating DOCK1 localization.

Nature Structural and Molecular Biology

Integration of the accelerator Aha1 in the Hsp90 co-chaperone cycle

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Heat-shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone that associates dynamically with various co-chaperones during its chaperone cycle. Here we analyzed the role of the activating co-chaperone Aha1 in the progression of the yeast Hsp90 chaperone cycle and identified a critical ternary Hsp90 complex containing the co-chaperones Aha1 and Cpr6. Aha1 accelerates the intrinsically slow conformational transitions of Hsp90 to an N-terminally associated state but does not fully close the nucleotide-binding pocket yet. Cpr6 increases the affinity between Aha1 and Hsp90 and further stimulates the Hsp90 ATPase activity. Synergistically, Aha1 and Cpr6 displace the inhibitory co-chaperone Sti1 from Hsp90. To complete the cycle, Aha1 is released by the co-chaperone p23. Thus, at distinct steps during the Hsp90 chaperone cycle, co-chaperones selectively trap statistically distributed Hsp90 conformers and thus turn Hsp90 into a deterministic machine.

Plant Journal

Temperature-dependent shade avoidance involves the receptor-like kinase ERECTA

SUMMARY
Plants detect the presence of neighbouring vegetation by monitoring changes in the ratio of red (R) to far- red (FR) wavelengths (R:FR) in ambient light. Reductions in R:FR are perceived by the phytochrome family of plant photoreceptors and initiate a suite of developmental responses termed the shade avoidance syn- drome. These include increased elongation growth of stems and petioles, enabling plants to overtop com- peting vegetation. The majority of shade avoidance experiments are performed at standard laboratory growing temperatures (>20°C). In these conditions, elongation responses to low R:FR are often accompanied by reductions in leaf development and accumulation of plant biomass. Here we investigated shade avoid- ance responses at a cooler temperature (16°C). In these conditions, Arabidopsis thaliana displays consider- able low R:FR-mediated increases in leaf area, with reduced low

R:FR-mediated petiole elongation and leaf hyponasty responses. In Landsberg erecta, these strikingly different shade avoidance phenotypes are accompanied by increased leaf thickness, increased biomass and an altered metabolite profile. At 16C, low R:FR treatment results in the accumulation of soluble sugars and metabolites associated with cold acclima- tion. Analyses of natural genetic variation in shade avoidance responses at 16C have revealed a regulatory role for the receptor-like kinase ERECTA.

Plant cell

The PP6 Phosphatase Regulates ABI5 Phosphorylation and Abscisic Acid Signaling in Arabidopsis

The basic Leucine zipper transcription factor ABSCISIC ACID INSENSITIVE5 (ABI5) is a key regulator of abscisic acid (ABA)?mediated seed germination and postgermination seedling growth. While a family of SUCROSE NONFERMENTING1-related protein kinase2s (SnRK2s) is responsible for ABA-induced phosphorylation and stabilization of ABI5, the phosphatase(s) responsible for dephosphorylating ABI5 is still unknown. Here, we demonstrate that mutations in FyPP1 (for Phytochrome-associated serine/threonine protein phosphatase1) and FyPP3, two homologous genes encoding the catalytic subunits of Ser/Thr PROTEIN PHOSPHATASE6 (PP6), cause an ABA hypersensitive phenotype in Arabidopsis thaliana, including ABA-mediated inhibition of seed germination and seedling growth. Conversely, overexpression of FyPP causes reduced sensitivity to ABA. The ABA hypersensitive phenotype of FyPP loss-of-function mutants is ABI5 dependent, and the amount of phosphorylated and total ABI5 proteins inversely correlates with the levels of FyPP proteins. Moreover, FyPP proteins physically interact with ABI5 in vitro and in vivo, and the strength of the interaction depends on the ABI5 phosphorylation status. In vitro phosphorylation assays show that FyPP proteins directly dephosphorylate ABI5. Furthermore, genetic and biochemical assays show that FyPP proteins act antagonistically with SnRK2 kinases to regulate ABI5 phosphorylation and ABA responses. Thus, Arabidopsis PP6 phosphatase regulates ABA signaling through dephosphorylation and destabilization of ABI5.