On the Inside

Nitric Oxide and Gravitopism

Nitric oxide (NO) is an endogenous signaling molecule implicated in a growing number of plant processes including plant defense, root initiation, stomatal closure in response to abscisic acid (ABA), salt tolerance, seed germination, nutrition, and flowering. As in mammalian cells, NO apparently acts in plants by switching on the enzyme guanylyl cyclase (GC), resulting in the accumulation of cyclic GMP (cGMP). Although NO is synthesized in roots, and both NO and cGMP have been shown to mediate auxin-induced root organogenesis, the roles of NO and cGMP in the responses of roots to gravistimulation have not been examined. In this issue, **Hu et al. (pp. 663–670)** demonstrate that gravistimulation of soybean (*Glycine max*) roots induces the accumulation of both NO and cGMP in the primary root tip. Fluorescence confocal microcopy revealed that the accumulation of NO was asymmetric, with NO being more concentrated in the lower side of the root. The application of NO to the lower side of horizontal roots enhanced gravitropic curvature, whereas application to the upper side suppressed it. Removal of NO with an NO scavenger or inhibition of NO synthesis via NO synthase (NOS) or nitrate reductase (NR) inhibitors reduced both NO accumulation and gravitropic bending, indicating that NO synthesis was required for the gravitropic responses and that both NOS and NR may contribute to the synthesis of the NO required. Auxin induced NO accumulation in root protoplasts and asymmetric auxin application to root tips resulted in asymmetric NO accumulation. Gravistimulation, NO, and auxin also induced the accumulation of cGMP, a response inhibited by removal of NO or by inhibitors of GC, compounds that also reduced gravitropic bending. Asymmetric NO accumulation and gravitropic bending were both inhibited by an auxin transport inhibitor and this inhibition of bending was overcome by treatment with NO or 8-bromo-cGMP, a cell-permeable analogue of cGMP. Together, these data indicate that NO and cGMP mediate auxin-induced gravitropic bending in soybean roots.

Making Arabidopsis an Isoprene Emitter

About one-third of all angiosperm species emit a significant fraction of recently fixed carbon as isoprene. Isoprene emission enhances plant tolerance to oxidants and rapid temperature change and is also a major source of natural air pollution. **Sharkey et al. (pp. 700–712)** have compared the genetics of the isoprene biosynthetic pathway of kudzu (*Pureria montana*), an isoprene emitter, with similar genes in Arabidopsis, a species that does not make isoprene. In the last step of isoprene biosynthesis, isoprene synthase converts dimethylallyl diphosphate, derived from the methylerythritol 4-phosphate (MEP) pathway, to isoprene. The MEP pathway genes in kudzu were similar to the corresponding genes in Arabidopsis. Phylogenetic analysis of the terpene synthase gene family indicated that isoprene synthases are either within the monoterpene synthase clade or sister to it. Two phenylalanine residues found exclusively in isoprene synthases make the active site smaller than other terpene synthase enzymes, possibly conferring specificity for the 5-carbon substrate rather than precursors of the larger isoprenoids. Expression of the kudzu isoprene synthase gene in Arabidopsis caused Arabidopsis to emit isoprene, indicating that a plant's ability to emit isoprene may depend, at least in the case of Arabidopsis, on whether or not it has a terpene synthase capable of using dimethylallyl diphosphate. Since isoprene-emitting plant species are phylogenetically scattered, it has been hypothesized that the trait of isoprene emission was lost many times during the course of evolution; the results reported here lend strong support to this hypothesis.

BIG and Root Architectural Responses to Low Phosphate

Low phosphorus (P) availability dramatically alters the spatial configuration of plant root systems by increasing root hairs, inhibiting primary root growth, and promoting lateral root formation. Such plastic root alterations play a crucial role in enabling the plant to explore increased soil volumes in search of nutrient rich patches. Despite several detailed anatomical studies on root architectural alterations in response to P limitation, little is known about the physiological and molecular mechanisms that coordinate these developmental changes. To gain insight into the regulatory mechanisms by which P availability alters postembryonic root development, **López-Bucio et al. (pp. 681–691)** performed a mutant screen to identify genetic determinants involved in the response of Arabidopsis to P deprivation. Three *low phosphate resistant root* lines (*lpr1-1* to *lpr1-3*) were isolated that demonstrated reduced lateral root formation under low P conditions. Genetic and molecular analyses revealed that all *lpr1* mutants were allelic to *BIG*, which is required for normal auxin transport and which the authors go on to show is required for pericycle cell activation prior to the formation of lateral root primordia under both high (1 mM) and low (1 μM) P conditions. *BIG*, however, does not appear to play a role in any of the other low P-induced architectural changes, including alterations in primary root growth, lateral root emergence, and root hair elongation. Exogenously supplied auxin restored normal lateral root formation in *lpr1* mutants in response to both P treatments. The application of brefeldin A, a fungal metabolite that blocks auxin transport, produced a root system phenotype similar to that observed in the *lpr1* mutants under both high and low P conditions. This finding suggests that BIG participates in vesicular targeting of auxin transporters. Taken together, these results point to a key role of *BIG* in pericycle cell activation to form lateral root primordia, a process that is not modified by P availability but that is required for increased lateral root emergence under low P conditions.

A Mitochondrial Mutator System in Maize

There are a growing number of examples of specific nuclear effects on

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particular components of plant mitochondrial genomes. The most well studied are the *Phaseolus vulgaris Fr* gene, whose dominant allele drastically reduces the copy number of a single mitochondrial subgenome that confers male sterility, and the *chm* mutation in Arabidopsis, which causes accumulation of characteristic rearrangements in mtDNA leading to maternally-conferred leaf variegation and distortion. In this issue, **Kuzmin et al. (pp. 779–789)** describe a novel type of maize (*Zea mays*) mutant with destabilized mitochondrial genomes. The destabilization is caused by recessive nuclear alleles in P2 popcorn-derived lines and leads to large-scale changes in mitochondrial genomes, some of which then become maternally inherited. Readily detectable changes in P2 mtDNA include: accumulation of numerous arrangements not seen previously at high levels; a significant decrease in the copy number of some normal regions; and multiple differences in the mtDNA profiles among sibling plants, as well as between parents and their progeny. Two types of events are apparently involved in the P2-specific destabilization of mitochondrial genome: the loss of nuclear control over the relative copy numbers of different mitochondrial subgenomes and a failure to transmit all of the subgenomes to progeny.

Tonoplast Aquaporins Facilitate NH_3 Transport

The physiological processes underlying the removal of ammonia and ammonium from the cytosol of plant cells are poorly understood. **Loqué et al. (pp. 671–680)** propose that the accumulation of ammonium in the acidic lumen of the vacuole is best explained by an acid-trap mechanism, in which the NH_3 diffusing across the vacuolar membrane subsequently binds a proton to form NH_4^+. But by what mechanism does NH_3 diffuse across the vacuolar membrane? By means of three different approaches, the authors provide evidence that indicates that the 2 tonoplast intrinsic proteins AtTIP2;1 and AtTIP2;3 mediate the transport of NH_3: (1) heterologous expression of both transporters in wild-type yeast (*Saccharomyces cerevisiae*) resulted in an enhanced tolerance against methylammonium, a toxic analog to ammonium; (2) expression of both AtTIPs in an ammonium uptake-defective yeast strain conferred yeast growth through ammonium uptake; and (3) expression of AtTIP2;1 in oocytes accelerated the transport of methylammonium. Transcript levels of both *AtTIPs* were increased by nitrogen (N) supply, but these increases did not follow those of the N-derepressed ammonium transporter gene *AtAMT1;1*. Transgenic Arabidopsis plants overexpressing *AtTIP2;1*, however, did not show altered ammonium accumulation in roots after ammonium supply, although *AtTIP2;1* mRNA levels in wild-type plants were up-regulated under these conditions. This lack of effect might be attributable to posttranslational modification of the protein. Although the researchers were unable to demonstrate unequivocally the physiological significance of AtTIP-mediated NH_3 transport in planta, they did show that AtTIP2;1 and AtTIP2;3 transport ammonium and methylammonium efficiently only at high medium pH and thus most likely represent NH_3 transporters. With their localization in the tonoplast and their transcriptional activation under ammonium supply, vacuolar TIP proteins are promising candidates to participate in the vacuolar loading of NH_3.

Arabidopsis Sphingosine Kinase

Sphingolipids are ubiquitous components of cellular membranes in eukaryotic cells. Sphingosine-1-phosphate (S1P) regulates many biological processes in mammals through its interactions with a family of specific cell surface G-protein-coupled receptors (GPCRs), and also serves as an intracellular second messenger in eukaryotes to regulate Ca^{2+} homeostasis, cell growth, and survival. In plants, S1P regulates guard cell behavior via Ca^{2+}-mobilization, inhibition of plasma membrane inwardly rectifying K^+ channels, and stimulation of slow anion channels. Recently, it has been shown that the enzyme responsible for S1P production, sphingosine kinase (SphK), is stimulated by ABA in guard cells of Arabidopsis and that S1P is effective in regulating guard cell turgor. Moreover, the action of S1P on ion channels is impaired in guard cells of Arabidopsis plants harboring T-DNA null mutations in the G-protein α-subunit gene, *GPA1*, suggesting that, as in mammals, heterotrimeric G-proteins are downstream targets for S1P in plants. In eukaryotes, cellular levels of S1P are ultimately regulated by the balance between synthesis via sphingosine kinase (SphK) and degradation by S1P lyase or phosphohydrolases specific for S1P. **Coursol et al. (pp. 724–737)** have characterized SphK from Arabidopsis leaves. SphK activity was mainly associated with the membrane fraction and phosphorylated predominantly the Δ4-unsaturated long-chain sphingoid bases sphingosine (Sph) and 4,8-sphingadienine. Evidence is presented that suggests that multiple isoforms of SphK may be expressed in Arabidopsis. Importantly, it was found that phytosphingosine-1-phosphate, a molecule similar to S1P, also regulates stomatal apertures and that its action is impaired in guard cells of Arabidopsis plants harboring T-DNA null mutations in the sole G-protein α-subunit gene, *GPA1*.

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