Nitrite Signaling

It is well known that nitrate is an important nutrient that supports plant growth and development. The application of nitrate also causes extensive changes in the expression of genes coding for proteins involved in nitrogen (N) metabolism. Indeed, genomic analyses have provided a comprehensive dataset of more than a thousand nitrate-responsive genes in Arabidopsis (Arabidopsis thaliana). Much less, however, is known about the signaling role of nitrite, the direct product of nitrate reduction. In this issue, Wang et al. (1735–1745) report that nitrite increases mRNA levels as quickly as nitrate in N-starved Arabidopsis roots. Both nitrite and nitrate inductions occur at concentrations as low as 100 nM. The response at low nitrite concentrations was not due to contaminating nitrate—a problem in several earlier studies. The speed of the response suggests that it is unlikely that reprovision of N is the cause of the nitrite response. Another possible mechanism is that nitrite is converted to nitric oxide, which elicits the response, but treatment with 250 µM nitrite revealed no increase in the fluorescence of roots stained with a nitric oxide-reactive dye. Transcriptome analysis using nitrate or nitrite showed that more than half of the nitrate-induced genes, which included genes involved in nitrate and ammonium assimilation, energy production, and carbon and N metabolism, responded equivalently to nitrite; however, the nitrite response was more robust and there were many genes that responded specifically to nitrite. Thus, it appears that nitrite can serve as a signal as well as if not better than nitrate.

Ethylene and Tylose Formation

Tyloses are outgrowths of xylem parenchyma cells into the lumens of adjoining vessels via vessel-parenchyma pits. Tyloses occur widely among plant species, and are induced by environmental stimuli such as wounding and pathogen infection. Tyloses impair xylem function by blocking vessels, but they are also a component in wound healing and may inhibit the intrusion of pathogens. Several hypotheses have been advanced to explain tylose initiation. One persistent idea is that tyloses form in response to the formation of air embolisms in xylem vessels. Like wounding and pathogen infection, natural senescence, heartwood formation, frost, and flooding also stimulate tylose development. Since each of these stimuli is also known to increase ethylene production, Sun et al. (pp. 1629–1636) were intrigued by the idea that ethylene may play a role in tylose formation. The authors have previously reported that there were no tyloses in intact stems of grape (Vitis vinifera) vines (Fig. 1), but that tyloses form rapidly in response to pruning. In this issue, the authors report that the pruning of actively growing grapevines was also followed by a 10-fold increase in the concentration of ethylene at the cut surface. When the pruning cut was made under water and maintained in water, embolisms were prevented but there was no reduction in the formation of tyloses or the accumulation of ethylene. Treatment of the stems with aminoethoxyvinylglycine, an inhibitor of ethylene biosynthesis, or silver thiosulfate, an inhibitor of ethylene action, delayed and greatly reduced the formation of tyloses in xylem tissue and the size and number of those that formed in individual vessels. These data are consistent with the hypotheses that wound ethylene production is the cause of tylose formation and that embolisms in vessels are not directly required for wound-induced tylosis in pruned grapevines.

The Two-Vacuoles Hypothesis Revisited

In the last decade, the notion that two different types of vacuoles can coexist with single plant cells has grown in popularity. These two types of vacuoles are commonly referred to as lytic vacuoles (LVs) that harbor hydrolytic enzymes and protein storage vacuoles (PSVs). In addition to proposed differences in luminal contents and sorting signals, it is widely held that these two types of vacuoles are also distinguishable from one another on the basis of the isoforms of tonoplast intrinsic proteins (TIPS) they possess. Thus, α-TIP is present in the tonoplast of the PSV in legume seeds, while γ-TIP is typical of LV and is highly expressed in elongating Arabidopsis root cells. A decade ago, a prominent paper reported that PSVs and LVs in the root tip of pea (Pisum sativum) and barley (Hordeum vulgare) seedlings are initially separate compartments that later fuse to form a central vacuole during cell elongation. Two papers now challenge these conclusions. Using antisera generated against various TIPs and storage proteins, Olbrich et al. (pp. 1383–1394) were unable to obtain evidence for separate vacuole populations in barley and pea roots. Instead, their observations point to the formation of a single type of hybrid vacuole containing storage proteins and having both α- and γ-TIPS in its tonoplast. As cells differentiate toward the zone of elongation, their vacuoles are characterized by increasing amounts of γ-TIP and decreasing amounts of α-TIP. Using Arabidopsis and a different methodological approach, Hunter et al. (pp. 1371–1382) also challenge previous claims of coexisting vacuole types within individual plant cells. These authors generated fusions between three Arabidopsis TIPs (α-, γ-, and δ-TIPs) and yellow fluorescent protein. They also produced soluble reporters consisting of red fluorescent protein and either the C-terminal vacuolar sorting signal of phaseolin or the sequence-specific sorting signal of

Figure 1. There are almost no tyloses in intact grapevine stems, but their rapid formation in response to pruning apparently involves the production of wound ethylene.

www.plantphysiol.org/cgi/doi/10.1104/pp.104.900242
proricin. In all the Arabidopsis vegetative organs studied, all TIP fusions localized to the tonoplast of the central vacuole and both of the luminal red fluorescent protein reporters were found within TIP-delimited vacuoles. In embryos from developing, mature, and germinating seeds, all three TIPs localized to the tonoplast of the PSVs. These results demonstrate that, in the Arabidopsis tissues analyzed, all vacuolar reporters localized to a common vacuolar location.

**Arabidopsides and Plant Defense**

The recognition by Arabidopsis roots of the *Pseudomonas syringae* effector proteins AvrRpt2 and AvrRpm1 induces the accumulation of very high levels of galactolipid-bound jasmonates (arabidopsides), including 12-oxophytodienoic acid (OPDA) and dinorphytodiеноic acid. In the present study, Kourtchenko et al. (pp. 1658–1669) examine arabidopside formation in Arabidopsis in response to pathogen invasion and wounding and provide new insights into the function of these compounds in plant cells. First, the authors demonstrate that the inactivation of the plastidial acyl transferase ACT1 does not inhibit the formation of arabidopsides after wounding or during the hypersensitive response. Second, the authors report that different signaling pathways lead to the formation of arabidopsides during the hypersensitive response and the wounding response, respectively. However, the formation of arabidopsides during both responses is dependent on an intact jasmonate signaling pathway. Additionally, the release of free jasmonates occurs in a time frame that overlaps with the observed reduction of arabidopside levels. The finding that arabidopsides accumulate in response to two different stimuli and that free 12-oxophytodiеноic acid does not simply follow the stream of “normal” fatty acids through the prokaryotic pathway seems to suggest that these compounds are more than just “accidental” by-products of hyperactive jasmonate synthesis. On the other hand, the apparent uniqueness of Arabidopsis as an arabi-
dopside-accumulating plant indicates that the role of the arabidopsides is dispensable or handled by other compound(s) in other plant species.

**Alkamide Action Involves Cytokinin Receptors**

Alkamides comprise more than 200 related compounds that have been found in as many as 10 plant families. Previous reports have indicated that alkamides, a class of plant-produced amino compound containing lipids, structurally related to N-acylthanolamides (NAEs), play a signaling role in plants. For example, amidenin, a non-substituted alkamide isolated from the actinomycete fungus *Amycolatopsis*, was shown to stimulate the growth of rice (*Oryza sativa*) seedlings and affinin, an alkamide present in the roots of *Heliopsis longipes*, was reported to alter the growth and development of the Arabidopsis root system. Thus, it was proposed that NAEs and alkamides might represent a new class of endogenous lipid signals that regulate plant development. López-Bucio et al. (pp. 1703–1713) identify the alkamide N-iso-butyl decanamide as the most active compound in inhibiting primary root growth and stimulating lateral root formation in Arabidopsis. They show that this compound affects cell division and differentiation. In leaves, the exogenous application of N-isobutyl decanamide was found to alter cell fate determination, leading to the production of ectopic blades along leaf petioles and vigorous outgrowths in the leaf lamina. In the root, these effects were accompanied with developmental tran-
sitions from lateral roots to callus-like structures. The involvement of cytokinins in mediating the observed activity of alkamides was tested using Arabidopsis cytokinin-signaling mutants lacking one, two, or three of the genes encoding the putative cytokinin receptors CRE1, AHK2, and AHK3. The triple cytokinin-receptor mutant was insensitive to N-isobutyl decanamide treatment. Taken together, these results suggest that alkamides and NAEs may belong to a class of endogenous signaling compounds that interact with a cytokinin-signaling pathway to control meristematic activity and differentiation processes.

**Actin Regulation of Hexokinase**

Glucose regulates many aspects of growth and function in plants, yeast, and mammals through both nutritive metabolic effects and gene regulatory mechanisms. In Arabidopsis, glucose can modulate the expression of almost 1,000 genes and many examples of cross talk between glucose and classical plant hormone signaling pathways have been noted. Independent of nutritional effects, plant glucose signaling interacts with other hormones to promote leaf expansion, vegetative growth, flowering, and senescence. In addition to phosphorylating glucose, hexokinase (HXK) functions in glucose sensing in many organisms. In this issue, Balasubramanian et al. (pp. 1423–1434) examine HXK’s role in glucose signaling in Arabidopsis. They show that mitochondrial-bound HXK can interact with actin and that a normal functioning actin cytoskeleton is required for HXK-dependent glucose signaling. Furthermore, plant glucose treatments result in rapid and extensive alterations in F-actin structure. These data suggest a role for the actin cytoskeleton in HXK-dependent glucose signaling in plants.

Peter V. Minorsky

Department of Natural Sciences

Mercy College

Dobbs Ferry, New York 10522