Hydrogen Sulfide: A New Node in the Abscisic Acid-Dependent Guard Cell Signaling Network?

Hydrogen sulfide (H$_2$S) is fast emerging as a new gaseous signaling molecule in organisms as diverse as bacteria, fungi, worms, humans, and plants (Kabil et al., 2014). Although H$_2$S has long been known to be toxic at high concentrations because of its ability to inhibit the mitochondrial cytochrome C oxidase, at much lower concentrations it has been proposed to act as a signal transmitter in conjunction with other reactive molecules, especially carbon monoxide, nitric oxide (NO), and reactive oxygen species (ROS; Lisjak et al., 2013; Hancock and Whiteman, 2014). Studies in various metazoan systems have established roles for H$_2$S in thermotolerance, atherosclerosis, inflammation, neural diseases, and diabetes (Hancock and Whiteman, 2014; Kabil et al., 2014).

Effects of H$_2$S on plants have been known since the 1960s, when H$_2$S was reported to influence the overall physiology of vegetative plants and to affect disease resistance (Rodriguez-Kabana et al., 1965; Joshi and Hollis, 1977). Only over the past 10 years, however, has H$_2$S been shown to modulate stress responses in plants, both biotic and abiotic (Hancock and Whiteman, 2014). Similar to its roles in metazoans, the overall action of H$_2$S is to relieve stress; however, the underlying molecular mechanisms remain largely unknown. Most studies in plants have taken a pharmacological approach, which inevitably makes interpretation of the observed responses difficult. However, an authentic l-Cys desulfhydrase, DES1 (At5g28030), was recently identified in Arabidopsis (Arabidopsis thaliana) that produces H$_2$S by l-Cys degradation (Alvarez et al., 2010). This discovery provides genetic tools to address some of the most outstanding questions related to H$_2$S generation, signaling, and its interactions with other reactive molecules in plants.

As a signaling molecule, H$_2$S is active on stomatal guard cells, as has been reported in multiple studies over recent years (García-Mata and Lamattina, 2013; Hou et al., 2013; Jin et al., 2013; Li et al., 2014). One of the key regulators of guard cells is abscisic acid (ABA), which is produced in response to a variety of stresses and promotes stomatal closure. An almost complete signaling pathway from ABA perception to gene regulation as well as ABA-dependent regulation of ion channel activities has been established in guard cells (Joshi-Saha et al., 2011). Furthermore, the roles of reactive small molecules, especially NO and ROS, have also been identified in the context of guard cell physiology and ABA signaling (García-Mata, et al., 2003; Gayatri et al., 2013; Song et al., 2014).

In this issue of Plant Physiology, Scuffi et al. (2014) utilize this knowledge of ABA signaling and the available genetic tools to validate the role of H$_2$S in guard cells as well as the cross talk between NO and H$_2$S in mediating stomatal responses. One of the key results of this work is the finding that DES1 activity is required for ABA-dependent stomatal closure. The authors show that in des1 knockout mutants, the stomata fail to close in response to ABA unless an H$_2$S donor is provided. These observations were confirmed by whole-plant analysis and were supported by expression analysis of ABA-regulated genes. Another important insight from the work relates to the relative position of H$_2$S and NO in guard cell signaling and their physiological interactions. Using a combination of pharmacological and genetic approaches, the authors demonstrate that the lack of endogenous NO significantly reduces the effects of H$_2$S on stomatal aperture and that NO acts downstream of H$_2$S to close stomata in ABA. These data address several discrepancies in the literature relating to H$_2$S and NO signaling in guard cells. Finally, the authors use the pyrabactin-resistant1 (pyr1)/pyrabactin-like1 (pyl1)/pyl2/pyl4 quadruple mutant and the Abscisic Acid-Insensitive1 (abi1-1) mutant of Arabidopsis, compromised in ABA perception and signaling, respectively, to show that H$_2$S also acts either downstream or in parallel with ABA perception, but nonetheless depends on the ABI1 protein phosphatase, much as was observed for NO.

These results not only establish H$_2$S as an important player in guard cell signaling, but they also open up exciting areas of research. For example, we can ask how the H$_2$S levels are regulated in a cell. There are other enzymes, in addition to Cys desulfhydrase, that are involved in sulfur metabolism. A spatially and temporally coordinated regulation of these enzymes will be required to maintain H$_2$S homeostasis. We also need to consider whether there might be additional DES1-like enzymes that are active in other cell types; the des1 mutants seem to show altered phenotypes only in guard cell-related responses. Interactions with H$_2$S should alter the sulfur oxidation states of its targets, and the identity of these targets, much less how H$_2$S might affect their activity/stability, is not known. H$_2$S does interact with other reactive small molecules such as NO and ROS (García-Mata and Lamattina, 2013). It is possible that these reactive molecules together maintain the redox status of a cell, thereby affecting overall stress responses. One such mechanism could be via the regulation of the mitochondrial electron transport chain in guard cells. An alternative oxidase present in guard cells has been proposed to act as a regulator of NO and ROS homeostasis (Cvetkovska et al., 2014). Plants expressing lower levels of alternative oxidase have high levels of NO and ROS and exhibit aberrant guard cell function and physiology. The enzyme acts by preventing the over-reduction of the components of the electron transport chain as well as

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allowing for efficient respiration even under the conditions when cytochrome C oxidase activity is diminished because of the high NO levels (Cvetkovska et al., 2014). An analysis of the effect of H₂S on mitochondrial respiration, in conjunction with ROS and NO, will be an interesting area of future research.

Key cellular processes affected by these gaseous molecules are now surfacing. For example, a group of plant-specific ethylene response factors (ERFs) have emerged as critical components for both NO and oxygen sensing in signal transduction (Gibbs et al., 2014). In the presence of NO, these ERFs are subjected to targeted proteolysis, which is likely to affect a multitude of cellular responses. These ERFs can also regulate other transcription factors such as ABA-Insensitive in ABA signaling and thereby act as potential hubs for integrating multiple exogenous and developmental cues. H₂S-mediated signaling may also integrate with a similar network of transcription factors. Such interactions, and the intersecting pathways they regulate, now need to be identified and detailed. Although these are challenging goals, especially given the complex chemistry and biology of H₂S, the work done by Scuffi et al. (2014) brings us a step closer to understanding the role of this enigmatic molecule in plant growth and fitness.

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