

Focus Issue: Calcium Signaling

It has been my pleasure to act as Guest Editor for this Focus Issue about calcium signaling. Ca^{2+} is the fifth most abundant element in the Earth's crust, yet high concentrations are cytotoxic because of precipitation of phosphates, proteins, and DNA. Homeostatic mechanisms to maintain low cellular Ca^{2+} evolved early, with the ability to pump Ca^{2+} ions out of the cytosol into the extracellular space being a common feature of cells (Case et al., 2007). Maintaining the 10,000-fold gradient of Ca^{2+} across the plasma membrane, the largest ionic gradient found in living cells, is energetically expensive; therefore, it was advantageous for cells to coopt the Ca^{2+} homeostatic machinery for signaling. Changes in the concentration of Ca^{2+} in the cytosol ($[\text{Ca}^{2+}]_{\text{cyt}}$) and other intracellular spaces participate in complex and dynamic signaling systems in all the kingdoms of life (Case et al., 2007). The Ca^{2+} signaling toolkit of ion channels, pumps, and regulatory mechanisms was present in the common ancestor of the plants and animals because many of the components are shared between modern animals and the chlorophyte algae. However, at some point in the transition from chlorophyte algae to land plants, selective pressure resulted in the loss of some components of the consensus toolkit, such as entire classes of Ca^{2+} channel and radiation of alternative mechanisms for Ca^{2+} signaling in the land plants (Wheeler and Brownlee, 2008). This Focus Issue concentrates on recent advances in the study of Ca^{2+} signaling in higher plants. The articles describe unique aspects of Ca^{2+} signaling associated with plant-specific functions in the cell and generated by plant-specific gene families. It is appropriate that a Focus Issue in *Plant Physiology* emphasizes the unique insights that arise from a community of researchers intent on unraveling the complexity of these dynamic signaling pathways in the most abundant kingdom.

My lifetime addiction to the calcium ion was cemented in a darkroom in the laboratory of Alistair Hetherington, then at Lancaster University, UK, watching in real time the first recording of a stimulus-induced $[\text{Ca}^{2+}]_{\text{cyt}}$ oscillation in a guard cell (McAinsh et al., 1995). In this issue, my laboratory reports that oscillating $[\text{Ca}^{2+}]_{\text{cyt}}$ signals are not restricted to guard cells, and the pattern of oscillation depends on both the cell and stimulus type, suggesting mechanisms for encoding specificity in signaling networks (Martí et al., 2013). Oscillatory changes in nuclear $[\text{Ca}^{2+}]$, induced by nodulation factors, were first described in 1996 by Ehrhardt et al. (1996). The ability of the nucleoplasm to support $[\text{Ca}^{2+}]$ oscillations independently of the cytosol has been considered controversial by some. In this issue, Charpentier and Oldroyd (2013) summarize the compelling evidence that the nucleoplasm can act as an independent compartment for Ca^{2+}

signaling and discuss the potential for decoding nucleoplasm Ca^{2+} signals. The mechanisms for decoding cytosolic Ca^{2+} signals are described in comprehensive Updates focusing on the calmodulins (Poovaiah et al., 2013), the plant-specific calmodulin-like proteins (Bender and Snedden, 2013), and calcium-dependent protein kinases (Schulz et al., 2013).

Ca^{2+} has been proposed to function in many of the major plant signaling pathways associated with biotic and abiotic stimuli (Dodd et al., 2010). It is very exciting that in this Focus Issue, there are reports of new roles for $[\text{Ca}^{2+}]_{\text{cyt}}$ signals in pathways associated with brassinosteroids (Zhao et al., 2013), yeast elicitors (Ye et al., 2013), and gravistimulation (Toyota et al., 2013). The last of these studies includes the astounding achievement of measuring intracellular ion concentrations in an aircraft performing parabolic flight.

The signaling pathways that bring about increases in $[\text{Ca}^{2+}]_{\text{cyt}}$ are the focus of Updates concerning the interrelationship of nitric oxide (Jeandroz et al., 2013) and reactive oxygen species (ROS), including a new model for long-distance signaling driven by ROS (Steinhorst and Kudla, 2013). New experimental data are presented that demonstrate that ROS activate Ca^{2+} channels to regulate other ion channels in the plasma membrane during abscisic acid (ABA)-induced stomatal movements (Wang et al., 2013a). A clever use of Ba^{2+} both as a blocker of K^{+} channel activity and as a carrier for charge-through Ca^{2+} channels, along with the presence of acetate to block Cl^{-} conductance, allowed Wang et al. (2013a) to measure ABA-induced Ca^{2+} channel activation at the plasma membrane in intact guard cells with microelectrodes. These data directly link plasma membrane Ca^{2+} channel activation to the PYR/PYL/RCAR ABA receptors. However, the *pyr1 pyl1 pyl2 pyl4* quadruple ABA receptor mutants are not completely insensitive to ABA because in the quadruple mutants, ABA is able to inhibit stomatal opening (Yin et al., 2013).

A consequence of the divergence of land plants from the chlorophyte algae is that the channels carrying Ca^{2+} across membranes can be different in plants and animals. The lack of a molecular identity for the major Ca^{2+} influx pathways has been an impediment to progress in plant signaling. New evidence identifying strong candidates for some of the influx pathways associated with Ca^{2+} signaling in plants, including the annexins, glutamate-like receptors, and cyclic nucleotide-gated channels (CNGCs), is summarized in an Update by Swarbreck et al. (2013). This Focus Issue also contains new evidence for roles for CNGC5 and CNGC6 in guard cells (Wang et al., 2013b) and CNGC2 and CNGC4 in pathogen defense and floral transition (Chin et al., 2013).

Many of the manuscripts presenting new data in this Focus Issue make use of the guard cell as an experimental system. Guard cells are an attractive tool for Ca^{2+}

signaling research in higher plants because of the low chlorophyll content permitting single-cell measurements of $[Ca^{2+}]_{cyt}$ using fluorescent reporters. Therefore, it is fitting that the last article to report is an excellent Update describing Ca^{2+} signaling mechanisms in guard cells (Laanemets et al., 2013). The hypothesis of Ca^{2+} priming, in which it is proposed that stress signals rapidly enhance $[Ca^{2+}]_{cyt}$ sensitivity, is developed, with suggestions for experiments to test this exciting new theory.

Most of the experimental studies collected in this Focus Issue employ genetic tools to unravel the complexities of Ca^{2+} signals. These studies are sorely needed because so many of the players remain unknown. However, the cell is a physiochemical system, and Ca^{2+} alterations are a rapid physiological process occurring on time scales much faster than genetic networks. Therefore, advances are also needed in cell physiology measurement techniques, and improved intracellular ion reporters are required to overcome the challenges of describing the beauty of Ca^{2+} signaling in walled, highly fluorescent cells.

I am grateful to all the authors for their excellent manuscripts and working under tight deadlines. The global plant Ca^{2+} signaling community is not large; thus, I am also immensely grateful to the band of anonymous reviewers who cheerfully accepted the burden of reviewing a large number of manuscripts in a short time frame.

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