COMMENTARY

Plant salt stress status is transmitted systemically via propagating calcium waves

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The existence and relevance of rapid long-distance signaling in plants is evident to any observer of the nastic movements of the Venus flytrap (*Dionaea muscipula*) or the sensitive plant (*Mimosa pudica*). However, all plants require the transmission of sensory information from the site of perception to other tissues to adjust their physiological states according to their environment. It is becoming increasingly apparent that rapid long-distance signals exist throughout the plant kingdom and may be responsible for initiating a multitude of physiological responses: electrical "action potentials" have been shown to convey wounding and salt-stress information from leaf-to-leaf (1, 2); a "hydraulic signal" transmitted by the direction of water movement within the xylem can mediate long-distance signaling of water stress experienced by the roots to the leaves in *Arabidopsis* (3); and reactive oxygen species (ROS) have been shown to propagate across a plant and carry stimulus-specific information to a variety of stresses (4). In PNAS, Choi et al. (5) use elegant approaches and present advances demonstrating that calcium can function as a long-distance signaling messenger, propagating in waves from roots and carrying salt-stress signals to induce expression of salt tolerance genes in leaves.

Calcium Waves Revealed

Choi et al. (5) expressed a genetically encoded FRET-based "cameleon" calcium sensor in the reference plant *Arabidopsis thaliana* and applied salt (NaCl) locally to seedling root tips (5). The authors observed calcium waves originating at the site of application and propagating to the shoot at a rate of 0.37–0.42 mm/s, requiring ∼2 min to travel the length of the plant.

Choi et al. (5) reveal that the NaCl-induced calcium wave could be blocked by application of drugs to the root that are known to inhibit plasma membrane calcium-permeable channels or calcium release pathways from intracellular organelles. Furthermore, they show that disruption of the *Two Pore Channel 1* (*TPC1*) gene results in a drastic 25-fold slowing of the salt-induced calcium wave, and overexpression of TPC1 increases the speed of the calcium wave by 1.7-fold. *TPC1* is a single gene in the *Arabidopsis* genome and encodes the slow vacuolar (SV) calcium-permeable ion channel (6). The calcium-permeable SV channels and *TPC1* orthologs are ubiquitous in land plants and have been found in the vacuolar membrane of all plant cell types analyzed to date. However, their biological functions have remained a matter of debate. The authors conclude with the tantalizing hypothesis that these channels may be required for stimulus-induced calcium waves in many plant biological processes.

The work by Choi et al. and other recent studies open an exciting new era in rapid long-distance plant signal transduction.

Unraveling the Function of the Ubiquitous Vacuolar SV Channels

Plant vacuoles occupy the majority of the volume of plant cells, and serve as a major intracellular calcium store. The SV channel represents the dominant conductance in plant vacuoles (7), and was shown to be activated by elevated cytosolic calcium (8). Subsequent research found that the SV channel is itself permeable to calcium ions (9, 10). Studies have suggested that diverse stimuli in plants cause intracellular calcium release from organelles (11). In animal cells "ryanodine receptor" channels in organelle membranes mediate calcium release into the cytosol in response to cytosolic calcium elevations, a process referred to as calcium-induced calcium release (CICR). These channels can produce propagating calcium waves (12). However, genome-sequencing efforts demonstrated that land plant genomes do not include homologs of ryanodine receptors, nor inositol 1,4,5 trisphosphate-gated calcium channels, showing that a different paradigm for organellar calcium release is required in plants. The finding that the ubiquitous SV channels are permeable to calcium ions led to the model that SV channels could be a distinct mechanism mediating CICR in plants (9).

However, a counter-hypothesis proposed that SV channels cannot mediate CICR (13). The reason for this counter-hypothesis lay in findings that the voltage dependence and vacuolar calcium dependence of SV channels in isolated vacuoles precludes steady-state ion fluxes from the vacuole to the cytoplasm (13, 14). Other evidence suggested that SV channels could mediate CICR (15). However, how could SV channels mediate calcium release? Two mechanisms are plausible. First, the typical free calcium gradient from vacuoles to the cytoplasm can be about 10,000-fold. Some studies have shown that shifts in the voltage dependence of SV channels can occur in response to hydrogen peroxide and lipid modulators (16–18). Because plant ion channels are hubs of many signaling pathways, it is conceivable that mechanisms exist that can slightly shift the voltage dependence to permit calcium release. However, direct biological evidence for SV channel-mediated CICR remains elusive. Second, experiments showed that calcium ions can flow freely, albeit transiently, from vacuoles to the cytoplasm during transient voltage shifts through so-called "tail currents" (19). These findings demonstrated that SV channels are not strictly unidirectional (diode-like) vacuole-directed cation channels. However, whether higher plant vacuoles undergo the necessary voltage shifts for this "transient" calcium release mode remains to be determined.

The finding that the TPC1 SV channel is required for long-distance calcium wave propagation (5) provides a physiological basis to investigate the precise roles that SV

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channels play in this response. In addition to a direct CICR function of SV channels, Choi et al. also consider a model that has presently not been excluded, in which SV channels may regulate a different calcium channel that produces the calcium wave. Further research will be needed to investigate this hypothesis and other possible models for TPC1/SV channel-dependent calcium wave propagation in plants.

Consequences of Altered Salt-Induced Calcium Wave Propagation

Choi et al. observed that following the calcium wave signal, many salt-induced genes are up-regulated in the leaves subsequent to salt application to the roots (5). To determine whether the calcium waves were required for up-regulation of these genes, the authors blocked these calcium waves by pharmacological or genetic means. They found that blockage of the Ca^{2+} waves correlates with the absence or reduction of expression of many of these salt-induced genes. Overexpression of TPC1, which accelerates the calcium wave propagation, results in an increase in expression of many of these same genes.

Finally, Choi et al. tested the sensitivity of plant growth on salt-containing agar plates (5). Interestingly, the authors found that TPC1-overexpressing lines show enhanced growth under salt stress compared to wild-type plants. Remarkably, the TPC1-overexpressing lines show enhanced growth even under nonsalt-stress, although to a lesser degree. The *tpc1* mutant plants did not show any salt sensitivity in this assay.

Open Questions and Future Outlook

The triggering of calcium waves in response to salt stress found by Choi et al. (5) is consistent with the findings of another recent study in plants using an independent bioluminescence resonance energy transfer-based calcium sensor (20). The existence of long-distance calcium signaling in plants opens up a variety of exciting new questions. For example, is the salt-induced calcium wave independent of the other characterized long-distance signaling mechanisms, or are they related? Some evidence exists that could link systemic ROS signaling with a calcium wave; calcium elevation activates NADPH oxidases (4), and ROS can induce calcium influx by activation of plasma-membrane calcium-permeable channels (21–23), providing a positive feedback mechanism for continued calcium/ROS wave propagation (4). Additionally, it remains to be determined which array of stimuli induces which types of long-distance signals, and how stimulus-specific information might be preserved.

Choi et al. showed that a *tpc1* knock-out allele greatly slows but does not abolish the calcium wave altogether, implying that more than one calcium channel must contribute to the salt stress-induced calcium wave (5). Furthermore, the likely plasma membrane calcium channel blocker, lanthanum, inhibits the calcium wave but does not block up-regulation of all salt-induced genes (5). Thus, a more complex model will need to emerge that accounts for distinct roles of calcium fluxes from different compartments and that provides a mechanism that links these signals together.

Relatively little is known regarding the perception events that initiate this systemic calcium wave in response to salt stress. Before the calcium wave propagation, salt stress induces a rapid calcium influx at the site of stress application (24). Choi et al. report that TPC1 largely does not influence this rapid initial salt-induced calcium influx, suggesting that TPC1 plays a distinct role for propagating the stress status signal (5) but not for perceiving the stress. Future work is needed to shed light on these earliest sensory mechanisms. Additionally, Choi et al. report that although many abiotic stresses induced a rapid calcium influx at the site of application, only salt stress resulted in a propagating calcium wave (5). How some calcium signals propagate while others do not will need to be determined in the future.

The work by Choi et al. (5) and other recent studies (2, 4) open an exciting new era in rapid long-distance plant signal transduction. The idea that plants are transmitting rapid systemic signals in response to stress is captivating, even if at first sight the end result isn't quite as dramatic as a plant that traps flies.

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