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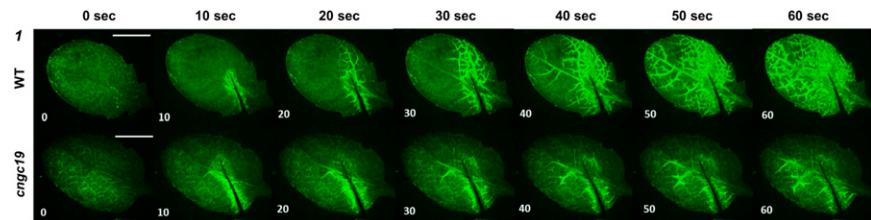
IN BRIEF

State of (In)flux: Action of a CNGC Ca²⁺ Channel in Defense against Herbivory^[OPEN]

Plants have evolved highly sophisticated signaling systems that enable them to coordinate growth and development and respond rapidly to environmental fluctuations. These long-range signals can take the form of mobile small molecules such as phytohormones and RNAs, but they can also be electrical signals associated with waves of calcium (Ca²⁺) and reactive oxygen species. The systemic signal is propagated to distal tissues extremely rapidly, at speeds ranging from ~100 to >1000 $\mu\text{m s}^{-1}$, which approaches rates of nerve conduction in animals (Choi et al., 2016).

In addition to its role in long-distance systemic signaling, elevated cytosolic Ca²⁺ ([Ca²⁺]_{cyt}) concentrations in the immediate vicinity of the stimulus site also demonstrate its involvement in local (within a single leaf) intercellular signaling. Ligand-gated cation channels in the plasma membrane permit Ca²⁺ influx into the cytosol, of which the cyclic nucleotide-gated channels (CNGCs) are known to be involved in Ca²⁺ fluctuations occurring in response to biotic and abiotic stress perception (reviewed in Meena and Vadassery, 2015). These Ca²⁺ signals are then spread to the surrounding tissue through the vasculature and trigger a multitude of defense responses. However, with regard to host defense specifically against herbivores, little is known about the precise mechanistic details of the transduction pathway involving local and long-distance Ca²⁺ signals. A new study by **Meena et al. (2019)** utilizing the common cutworm herbivore *Spodoptera litura* describes the role of a member of the Arabidopsis (*Arabidopsis thaliana*) CNGC family, CNGC19, in herbivory-induced local Ca²⁺ influx and downstream defense responses.

Several CNGCs show altered expression in response to mechanical wounding and/or treatment with *S. litura* oral secretion.



Wounding Triggers the Rapid Induction and Propagation of Cytosolic Ca²⁺ Levels.

Wild-type Arabidopsis leaves expressing the GCaMP3 fluorescent protein-based Ca²⁺ sensor show a local increase in [Ca²⁺]_{cyt} within 10 s of mechanical wounding that is then transmitted across the leaf surface via the vasculature. This Ca²⁺ signal propagation is severely disrupted in *cngc19* loss-of-function mutants. WT, wild type. Adapted from Meena et al. (2019) Figure 2.

However, CNGC19 is highly and rapidly upregulated in both local and systemic leaves, indicating that this isoform is specifically involved in herbivory perception responses. In the presence of cyclic nucleotides, CNGC19 functions as a selectively permeable Ca²⁺ ion channel that localizes exclusively to the plasma membrane.

To characterize the role of CNGC19 in the generation and propagation of the Ca²⁺ signal during wounding, Meena and co-workers utilized the GCaMP3 fluorescent protein-based [Ca²⁺]_{cyt} sensor. Following wound-induced activation of CNGC19 in the wild-type plants, the Ca²⁺ signal was seen to spread rapidly from the wound site to the vasculature and then across the leaf itself (see figure). It has previously been hypothesized that this latter phase of Ca²⁺ signal transmission may occur via the plasmodesmata (Toyota et al., 2018) and is thus slower. Crucially, however, in *cngc19* loss-of-function mutants this local Ca²⁺ signal propagation was significantly impaired.

Concomitant with its role in regulating herbivory-induced Ca²⁺ fluxes, CNGC19 was additionally found to positively regulate other aspects of downstream induced defense responses to *S. litura*. Analysis of *cngc* loss-of-function mutants revealed that they were more susceptible to caterpillar feeding in comparison to the wild type. These mutants also accumulated

significantly lower levels of jasmonic acid (JA) and its biologically active derivative jasmonoyl-isoleucine during herbivore attack, accompanied by the downregulation of known JA-responsive marker genes. Moreover, expression of CNGC19 was shown to be dependent on the jasmonate receptor CORONATINE INSENSITIVE1; thus, CNGC19 appears to function as part of a positive feedback loop.

Since JA biosynthesis is rapidly induced upon wounding and is known to be regulated by Ca²⁺ cascades (Wasternack and Hause, 2013), the attenuated Ca²⁺ signal propagation in the *cngc19* mutants likely contributes to this observed *cngc19* defense phenotype. Surprisingly, the *cngc19* mutants also exhibit decreased accumulation of aliphatic glucosinolates—a major group of secondary metabolites active in plant resistance to chewing insects—but increased levels of its precursor, methionine. Expression levels of branched-chain aminotransferase4 (*BCAT4*), which catalyzes the first step in the aliphatic glucosinolate biosynthetic pathway, were significantly downregulated in the *cngc19* lines, and *bcat4* mutants pheno-copied *cngc19* mutants in insect feeding assays. Taken together, the Ca²⁺ CNGC19 channel likely regulates *BCAT4* expression and thus the biosynthesis of defense-related secondary metabolites, further implicating CNGC19

as a positive regulator of induced herbivore immune responses.

This study highlights the role of one specific CNGC channel in local Ca^{2+} signal propagation in response to herbivory. The glutamate receptor-like (GLR) proteins are another well-studied group of ion channels shown to be essential in wound-induced electrical and Ca^{2+} signaling events (Toyota et al., 2018). It would therefore be of interest to investigate any potential crosstalk between glutamate receptor-like proteins, CNGCs, and other groups of ion channels involved in generating these Ca^{2+} fluxes. Such rapid local and long-distance Ca^{2+} signals, coupled with reactive oxygen species and electrical signals, also occur under other biotic and abiotic stress conditions. How these multiple signals are integrated by the plant to

generate an appropriate stimulus-specific response remains to be determined.

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