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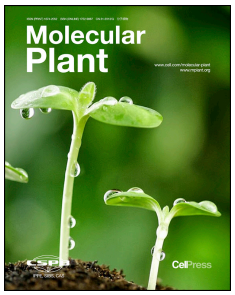

Calcium signaling primes RNA interference during viral infection

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Calcium signaling primes RNA interference during viral infection

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Plants resist viral infection through multiple mechanisms. These include physical barriers, activation of NUCLEOTIDE-BINDING LEUCINE-RICH REPEAT PROTEINS (NLRs), autophagy, biosynthesis of defence-related phytohormones and RNA interference (RNAi). RNAi supresses the expression of viral RNA and is conserved across several eukaryotic kingdoms. During infection, RNA-DEPENDENT RNA POLYMERASES (RDRs) amplify viral RNA fragments into double-stranded RNAs (dsRNAs). DICER-LIKE ENDORIBONUCLEASES (DCLs) then cleave these dsRNAs into small interfering RNAs (siRNAs), which associate with RNASE H-LIKE ARGONAUTES (AGOs) and form RNA-INDUCED SILENCING COMPLEXES (RISCs). SiRNAs guide RISCs to cleave homologous viral RNA fragments and repress viral replication (Fang and Qi, 2016). While RNAi has been extensively studied for the past few decades, the initial cues that prime the expression of RNAi-related genes during viral infection were unclear. A recent study by Liu and colleagues sheds new light on the regulation of RNAi during the early stage of infection (Wang et al., 2021).

Activation of both cell surface and intracellular immune receptors lead to cytosolic calcium (Ca^{2+}) influx, which activates the Ca^{2+} sensor CALMODULIN (CaM) and transcription factors such as CAM-BINDING TRANSCRIPTION ACTIVATOR-3 (CAMTA3) (Du et al., 2009). Wang et al showed that mechanical wounding and aphid injury lead to both Ca^{2+}- and CaM-dependent activation of CAMTA3, which regulates the expression of multiple RNAi-related genes, including RDR6, AGO1/2 and DCL1 (Wang et al., 2021). While CAMTA3 directly binds the promoter of RDR6, expression of AGO1/2 and DCL1 are regulated indirectly. The authors showed that BIFUNCTIONAL NUCLEASE-2 (BN2) degrades the microRNAs (miRNAs) that silence AGO1/2 and DCL1. Activation of CAMTA3 leads to upregulation of BN2, which indirectly derepresses the silencing of AGO1/2 and DCL1. Importantly, both CAMTA3 and BN2 are required for resistance against multiple plant viruses. In addition, Wang

et al discovered that V2 protein of two geminiviruses *Cotton leaf curl Multan virus* (CLCuMuV) and *Tomato yellow leaf curl China virus* disrupt the interaction between CaM and CAMTA3 to supress Ca^{2+}-mediated RNAi priming, revealing how this antiviral defence mechanism can be counteracted by plant viruses (Wang et al., 2021) (Figure 1).

This study demonstrates that wounding-induced Ca^{2+} influx (likely triggered by pattern-recognition receptors) primes RNAi in plants during the onset of viral infection. This implies that RNAi can be primed by the activation of both cell surface- and intracellular immune receptors through CaM and CAMTA3. Moreover, *geminiviruses* target this signalling pathway as a counter-defence mechanism to suppress RNAi priming. Future studies are needed to understand the role of calcium signaling in antiviral silencing in the absence of wounding during later stages of systemic viral infection (Wang et al., 2021).

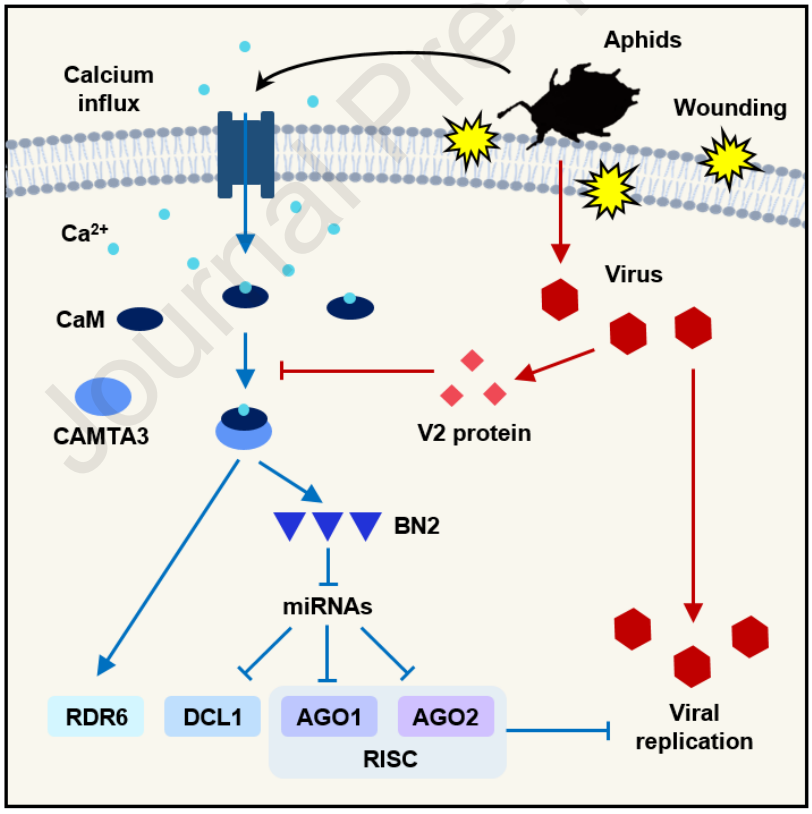

Figure 1. Regulation of proteins involved in RNAi via CAMTA3. Insect feeding and mechanical wounding induce cytosolic calcium influx, which activates CaM and CAMTA3. Activation of CAMTA3 leads to the upregulation of proteins involved in RNAi, including RDR6, DCL1 and AGO1/2. RNAi represses viral replication and V2 protein from *geminivirus* supresses the association between CaM and CATMA3 to derepress this process.

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