HHS Public Access

Author manuscript

Cell Host Microbe. Author manuscript; available in PMC 2022 September 08.

Published in final edited form as:

Cell Host Microbe. 2021 September 08; 29(9): 1339–1341. doi:10.1016/j.chom.2021.08.008.

Ca^{2+} kickstarts antiviral RNAi

April DeMell[1], **Savithramma P. Dinesh-Kumar**[1,*]

[1]Department of Plant Biology and The Genome Center, College of Biological Sciences, University of California, Davis, Davis, CA 95616, USA

Abstract

RNAi is a major plant antiviral strategy. However, the mechanisms triggering RNAi are unknown. In this issue of *Cell Host & Microbe*, Wang et al. (2021) demonstrate that wound-induced calcium signaling induces RNAi activation. As a counter-defense, a viral suppressor of RNAi (VSR) interferes with players in calcium signaling.

Plant viruses must overcome physical hurdles including the cuticle and cell wall for successful invasion of the host, which often occurs via vector transmission or mechanical damage. Host wounding stimulates fluctuations in calcium (Ca^{2+}) concentrations, resulting in the induction of Ca^{2+}-mediated signaling pathways, which are critical in early defense responses. These pathways utilize Ca^{2+} sensors such as calmodulin (CaM), which upon Ca^{2+} binding, undergoes conformational changes and interacts with downstream targets. These targets are often regulatory transcription factors, such as CaM-binding transcription activators (CAMTAs), which have roles in development, growth, and defense (Iqbal et al., 2020). Specifically, CAMTA3 has been demonstrated to modulate the expression of genes involved in biotic and abiotic stresses. The rapid stress response element (RSRE) in the promoter regions functions as the transcriptional binding site for CAMTA3, making it particularly important for early defense responses. Previously, CAMTA3's involvement in pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), effector-triggered immunity (ETI), and systemic acquired resistance (SAR) have been characterized and involves repressional regulation of important defense genes such as *PR1, EDS1, EIN3*, and *Chitinase* (Iqbal et al., 2020). However, no link has previously been described between the Ca^{2+}-CaM-CAMTA3 signaling cascade and the major form of antiviral defense known as RNA silencing (RNAi).

In addition to the widely known PTI and ETI branches of plant immunity, an equally important branch of immunity is RNAi. While RNAi has traditionally been defined as a critical defense against diverse plant viruses, recent evidence has demonstrated that it also has an important role against non-viral pathogens, including fungi and bacteria (Muhammad et al., 2019; Pumplin and Voinnet, 2013). This eukaryotically conserved form of post-transcriptional gene silencing (PTGS) is activated in response to the double-stranded RNA (dsRNA) or highly structured single-stranded RNA (ssRNA) that results from normal virus replication. After recognition, the viral RNAs are processed by a Dicer--like (DCL)

*Correspondence: spdineshkumar@ucdavis.edu.

protein into virus-derived small-interfering RNAs (vsiRNAs). Following methylation, a vsiRNA is loaded into the RNA-induced silencing complex (RISC), of which an Argonaute (AGO) protein forms the central catalytic unit. Utilizing a "search and scan" method, the vsiRNA-guided complex uses complementary base-pairing to target the invading viral genome for degradation. Enhancing the value of this system, RNA-dependent RNA polymerases (RDRs) can amplify these vsiRNAs, allowing the RNAi signal to amplify and spread to neighboring cells and, ultimately, throughout the plant. However, plants encode multiple DCLs, AGOs, and RDRs. Also, factors such as virus type and plant species can impact which RNAi components are predominantly utilized. For example, during infection by DNA-based geminiviruses, DCL1–4 and RDR6 are mainly employed for RNAi. Contrastingly, during RNA virus defense, DCL2 and DCL4 play the primary roles in RNAi during potyviral infection, and both RDR1 and RDR6 are important during cucomovirus infection (Muhammad et al., 2019; Pumplin and Voinnet, 2013).

In an effort to protect themselves from RNAi-mediated defense, viruses encode viral suppressors of RNAi (VSRs) that are capable of subduing RNAi machinery and related components through diverse mechanisms (Muhammad et al., 2019; Pumplin and Voinnet, 2013). For example, the P19 VSR from a tombusvirus induces expression of miR168, which affects AGO1 mRNA stability and translation. Additionally, the P38 coat protein of Turnip crinkle virus (TCV) directly binds to AGO1 and AGO2 and affects small interfering (si)RNAs loading (Pumplin and Voinnet, 2013). Moreover, VSRs can target non-RNAi forms of plant resistance, including PTI and ETI (Nicaise and Candresse, 2017).

Since defense-related processes can be energetically costly and are often integrated with developmental pathways, induction must be tightly regulated. Indeed, expression of many defense-related genes, including those involved in Ca^{2+} signaling and RNAi, are activated upon plant perception of an invading virus. For example, CAMTAs are among the earliest induced genes after infection by specific strains of a potyvirus and a cucomovirus in tobacco (Iqbal et al., 2020). Additionally, CAMTA3 is highly expressed in maize during infection with a fijivirus (Iqbal et al., 2020). But what is the initial signal that primes RNAi to respond to early viral infection? How is RNAi machinery maintained during normal conditions, then initiated, sustained, and amplified under stress conditions? In a new report by Wang and colleagues in this issue of *Cell Host & Microbe*, these important questions were explored, and a relationship between the Ca^{2+}-CaM3-CAMTA3 signaling cascade and antiviral RNAi is uncovered (Wang et al., 2021).

Wang and colleagues revealed that wounding-induced Ca^{2+} fluxes activate the interaction of CaM3 and CAMTA3 in *Nicotiana benthamiana* plants, ultimately triggering increased expression of antiviral RNAi-related genes including DCL1, BN2, AGO1, AGO2, and RDR6 (Wang et al., 2021). After CaM3 activation, the CAMTA3 transcription factor induces expression of RDR6 and the ribonuclease BN2 via direct binding to the promoters of these genes. However, since the promoters of DCL1, AGO1, and AGO2 do not possess the required binding sites, how are they induced by CAMTA3 activation? Using a combination of transcriptomic and reverse genetic tools, BN2 was identified as a potential culprit. BN2 degrades the micro (mi)RNAs that target the key RNAi components DCL1, AGO1, and AGO2, alleviating their suppression. To tie it all together, the authors demonstrate the

requirement of CAMTA3 for wounding-mediated suppression of multiple DNA and RNA viruses. Taken together, the data presented demonstrate that plant RNAi is regulated by Ca^{2+}-signaling and that mechanical and insect vector wounding is a putative, initial trigger that kickstarts the antiviral RNAi pathway.

Although most VSRs are known to directly target RNAi machinery, their interference with Ca^{2+}-signaling components is largely unknown. Regulator-of-gene-silencing calmodulin (rgs-CaM) functions as an endogenous RNAi suppressor and is co-opted by VSRs from multiple viruses to do their bidding (Anandalakshmi et al., 2000; Li et al., 2017; Nakahara et al., 2012). However, rgs-CaM also targets VSRs for degradation through the autophagy process, indicating that rgs-CaM may also function to remove VSRs (Nakahara et al., 2012). Although these findings seem contradictory, rgs-CaM may degrade itself to impede VSRs. Irrespective of how rgs-CaM functions in antiviral defense, its role in Ca^{2+}-signaling has not been reported.

Wang et al. demonstrate that CaMs involved in Ca^{2+}-signaling positively regulate RNAi and that the geminiviral V2 VSR disrupts the CaM3-CAMTA3 regulatory complex by directly interacting with CaM3. This discovery represents one of the first examples of an authentic CaM involved in Ca^{2+}-signaling being targeted by a VSR. As previously described, the engagement of a specific DCL, AGO, or RDR is situational and can differ based on the viral target. While Wang et al. show that CaM3-CAMTA3 regulates DCL1, AGO1/2, and RDR6, could other CaMs/CaM-like proteins or other transcription factors besides CAMTA3 be involved as well? Could the Ca^{2+} -signaling components that are being exploited change depending on the type of the viral invader? Do other VSRs target CaMs that associate with CAMTA-like transcription factors to interfere with Ca^{2+}-mediated defense signaling?

Traditional dogma has often painted the plant immune system as a few dominant, independent branches. However, mounting evidence indicates that pathways are more mechanistically intertwined, possessing interdependencies and common players between branches like PTI, ETI, Ca^{2+}-signaling, and RNAi that are essential for their initiation and potentiation (Pruitt et al., 2021). In this vein, Wang et al. introduce us to some of these intertwined branches, connecting the damage response and $Ca2^{+}$-signaling with RNAi, a signaling cascade mediated by multitasking molecular receptors and relays that are also involved in other key immunological branches. Ultimately, a greater understanding of the relationships between different immunological pathways could be transformative for our understanding of plant immunity.

ACKNOWLEDGMENTS

Immunity-related work in SPD-K laboratory is supported by NIH grant GM132582.

REFERENCES

Anandalakshmi R, Marathe R, Ge X, Herr JM Jr., Mau C, Mallory A, Pruss G, Bowman L, and Vance VB (2000). A calmodulin-related protein that suppresses posttranscriptional gene silencing in plants. Science 290, 142–144. 10.1126/science.290.5489.142. [PubMed: 11021800]

Iqbal Z, Shariq Iqbal M, Singh SP, and Buaboocha T (2020). Ca(2+)/calmodulin complex triggers CAMTA transcriptional machinery under stress in plants: Signaling cascade and molecular regulation. Front. Plant Sci 11, 598327. 10.3389/fpls.2020.598327. [PubMed: 33343600]

Li F, Zhao N, Li Z, Xu X, Wang Y, Yang X, Liu SS, Wang A, and Zhou X (2017). A calmodulin-like protein suppresses RNA silencing and promotes geminivirus infection by degrading SGS3 via the autophagy pathway in Nicotiana benthamiana. PLoS Pathog. 13, e1006213. 10.1371/journal.ppat.1006213. [PubMed: 28212430]

Muhammad T, Zhang F, Zhang Y, and Liang Y (2019). RNA Interference: A natural immune system of plants to counteract biotic stressors. Cells 8, 38. 10.3390/cells8010038.

Nakahara KS, Masuta C, Yamada S, Shimura H, Kashihara Y, Wada TS, Meguro A, Goto K, Tadamura K, Sueda K, et al. (2012). Tobacco calmodulin-like protein provides secondary defense by binding to and directing degradation of virus RNA silencing suppressors. Proc. Natl. Acad. Sci. USA 109, 10113–10118. 10.1073/pnas.1201628109. [PubMed: 22665793]

Nicaise V, and Candresse T (2017). Plum pox virus capsid protein suppresses plant pathogen-associated molecular pattern (PAMP)-triggered immunity. Mol. Plant Pathol 18, 878–886. 10.1111/mpp.12447. [PubMed: 27301551]

Pruitt RN, Gust AA, and Nürnberger T (2021). Plant immunity unified. Nat. Plants 7, 382–383. 10.1038/s41477-02100903-3. [PubMed: 33785867]

Pumplin N, and Voinnet O (2013). RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence. Nat. Rev. Microbiol 11, 745–760. 10.1038/nrmicro3120. [PubMed: 24129510]

Wang Y, Gong Q, Wu Y, Huang F, Ismayil A, Zhang D, Li H, Gu H, Ludman M, Fátyol K, et al. (2021). A calmodulin-binding transcription factor links calcium signaling to antiviral RNAi defense in plants. Cell Host Microbe 29, 1393–1406.e7. 10.1016/j.chom.2021.07.003. [PubMed: 34352216]