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JAcked Responses Go Viral: Hormonal Regulation of Antiviral RNAi

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Little is known about the mechanism that regulates the core steps of antiviral RNA interference (RNAi) pathway in plants and animals. In this issue of *Cell Host & Microbe*, Yang et al. (2020) provide compelling evidence for the regulation of antiviral RNAi by the jasmonate hormone signaling in plants.

Hormones are signal molecules that regulate growth and development as well as the immune responses to microbial infection. Among a variety of important hormones produced by plants, for example, salicylic acid (SA) acts as a major plant defense hormone to control the expression of a large number of genes with well-defined functions in the defense against bacterial and fungal pathogens (Zhou and Zhang, 2020). The RNA interference (RNAi) pathway directs a broadly conserved virus resistance mechanism in plants and animals (Guo et al., 2019). Studies in the past two decades have extensively characterized the function and mechanism of the key genes that mediate antiviral RNAi in model host species such as *Arabidopsis thaliana*, *Caenorhabditis elegans*, and *Drosophila melanogaster* (Guo et al., 2019) (Figure 1). Recent studies began to identify genes with a regulatory role in antiviral RNAi. For example, Argonaute-18 (AGO18) potently enhances antiviral RNAi in rice plants by sequestering microRNA 168 (miR-168), which represses the expression of a key component of the antiviral RNA-induced silencing complex (RISC), AGO1 (Wu et al., 2015). Genetic screens in *A. thaliana* have led to the identification of specific lipid flippases that promote the amplification of virus-derived small interfering RNAs (siRNAs) in *Arabidopsis* plants (Guo et al., 2017). In a paper published in this issue of *Cell Host & Microbe*, Yang et al. (2020) provide evidence for the regulation of antiviral RNAi by a major hormone of host plants, jasmonate (JA).

JA signaling activates a potent plant defense response to insect herbivores (Wang et al., 2019). JA perception by the F-box protein COI1 triggers ubiquitination and degradation of JA ZIM-domain proteins (JAZ), releasing the JAZ-bound transcription factors such as MYB proteins to activate the expression of JA-responsive genes (Figure 1). By examining rice plant infection with rice stripe virus (RSV), Yang and colleagues have demonstrated AGO18 as a JA-responsive gene regulated specifically by COI1, JAZ6, and MYB protein JAMYB in the JA pathway (Figure 1).

Yang and colleagues find that AGO18 promoter contains JAMYB-binding AGAT motifs and is induced by JA treatment in wild-type rice seedlings, but not in JAMYB-knockout mutant seedlings. They showed that JAZ6 interacts with JAMYB inside plant cells and suppresses JAMYB-dependent activation of AGO18 promoter. Importantly, JA application enhances RSV resistance and RSV replicates to lower titers in JAZ6-knockout mutant plants, but to higher titers in *coi1* and *jamyb* mutant plants than wild-type rice plants. These findings demonstrate that JA signaling positively regulates antiviral defense in rice plants.

Yang et al. (2020) further show that RSV infection enhances JA accumulation and induces the expression of key JA pathway genes in wildtype rice plants. They also revealed that the induction of AGO18 by RSV is compromised in *coi1* mutant plants. However, it is currently unknown whether AGO18 activation in response to RSV infection is compromised and enhanced, respectively, in *jamyb* and *jaz6* mutant rice seedlings. Nevertheless, Yang and colleagues found that stable overexpression of JAMYB enhances virus resistance in the wildtype, but not *ago18* mutant background. Moreover, *jamyb* and *ago18* double mutant plants are not more susceptible to RSV infection than *jamyb* or *ago18* single mutants. These results provide further support that the antiviral function of JA signaling is mediated specifically by AGO18.

Notably, Yang and colleagues found that stable transgenic expression of RSV coat protein (CP) alone confers RSV resistance in wildtype rice plants, but neither *coi1* nor *ago18* mutant plants. Earlier work from the same group has identified RSV CP as the viral elicitor to induce AGO18 expression (Wu et al., 2015). In this study, they show that transgenic expression of CP enhances JA accumulation in wild-type rice plants and that CP-induced AGO18 activation becomes compromised in *coi1* mutant plants. Together, these findings demonstrate that CP-induced JA signaling is sufficient to confer antiviral defense by AGO18.

It is known that transgenic expression of diverse plant viral CP genes confers specific virus resistance by either constitutive RNAi to target the viral RNAs or mechanisms dependent on the expression of CP (Callaway et al., 2001). Therefore, the engineered resistance described by Yang and colleagues might be mediated by an entirely distinct mechanism. Future studies should verify whether the resistance is both broad-spectrum and dependent on the expression of CP. In this regard, it is important to note that *ago18* mutant plants exhibit enhanced disease susceptibility not only to RSV, which contains a segmented single-stranded RNA genome using an ambisense coding strategy, but also to rice

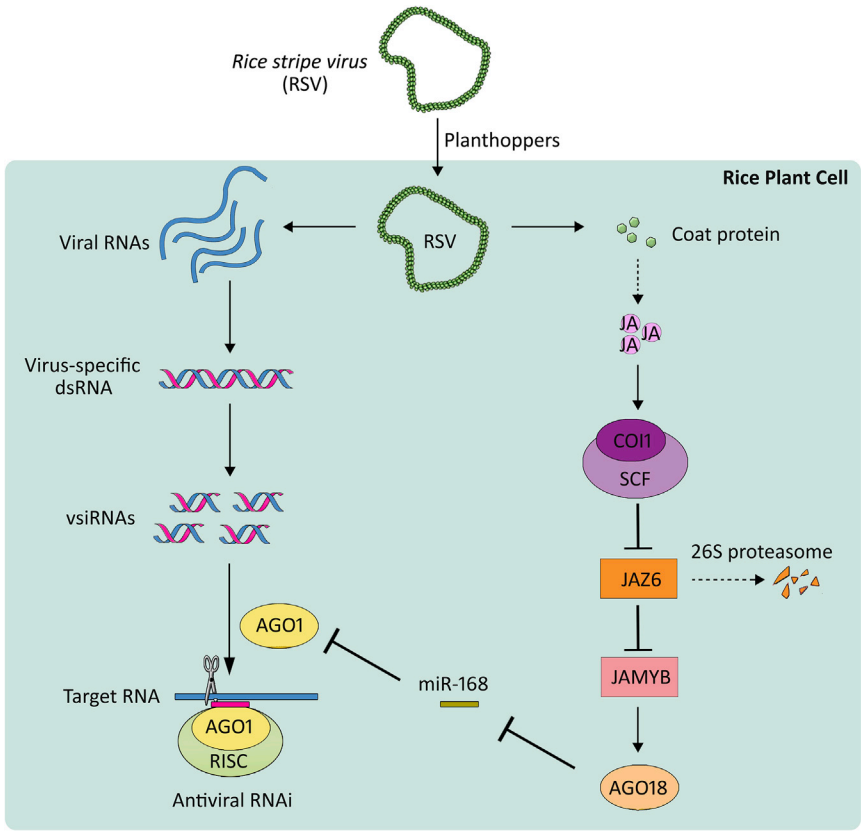

Figure 1. Regulation of antiviral RNAi by hormone jasmonate in rice plants

Virus-specific dsRNA is recognized as a pathogen-associated molecular pattern (PAMP) by the type 3 endonuclease Dicer as a host pattern recognition receptor (PRR) and further processed into virus-derived siRNAs. These host-produced viral siRNAs (vsiRNAs) function as the specificity determinants to direct antiviral RNAi in RNA-induced silencing complex (RISC), of which AGO1 is a key effector molecule. In healthy rice plants, JAZ6 interacts with transcription factor JAMYB to repress the transcription of *AGO18*. Upon infection with RSV, expression of the viral coat protein (CP) enhances production of JA by an unknown mechanism, thereby activating JA signaling. JA perception by COI1 triggers COI1-mediated degradation of JAZ6, releasing JAMYB to bind to the promoter of *AGO18* gene and to activate *AGO18* expression. Specific sequestration of miR-168 by AGO18 alleviates the repression of *AGO1* expression by miR-168, thus upregulating AGO1-dependent antiviral RNAi.

dwarf virus with a segmented double-stranded RNA genome (Wu et al., 2015). Moreover, Yang and colleagues found that compared with RSV infection of wildtype plants, transgenic CP expression significantly enhances symptom severity of RSV in *ago18* mutant plants, but not *coi1* mutant plants, suggesting an intriguing activity of the CP transgene to promote disease development in the absence of AGO18.

RSV is transmitted in the field only by viruliferous small brown planthoppers, unlike many plant viruses that are spread by contact between diseased and healthy leaves. Thus, an attractive hypothesis is that the observed upregulation of antiviral RNAi by the JA pathway with a function specialized in insect resistance has evolved in a co-adaptation of plants to arthropod-borne viruses. Nevertheless, Yang and colleagues have convincingly illustrated the importance of RSV infection in the activation of JA signaling and AGO18 expression given that virus-free planthoppers have been used as controls in virus inoculation. Moreover, previous studies have documented the interactions of the JA pathway with viruses that are transmissible by mechanical means and/or arthropods (Lewsey et al., 2010; Westwood et al., 2014). For example, cucumber mosaic virus encodes an RNAi suppressor protein 2b that also actively inhibits JA signaling by directly interacting with and repressing JA-induced degradation of host JAZ proteins (Wu et al., 2017).

In conclusion, this body of work provides an example for the regulation of antiviral RNAi by a major hormone of the host, which opens up a new avenue to investigate the hormonal control of antiviral RNAi. For example, it will be interesting to examine whether some of the known genes regulated by JA, SA, and other plant hormones (Zhou and Zhang, 2020) act in the antiviral RNAi pathway. It should be pointed out that AGO18 is a member of the Argonaute protein clade specific only to monocotyledonous plants and functions to regulate the expression of a core component of the RNAi machinery. Thus, further studies are necessary to determine whether JA signaling also regulates the function of antiviral RNAi in dicotyledonous plants that do not encode a member in the AGO18 clade.

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Listening In: Plasmacytoid DC, Monocyte-Derived DC, and Neutrophil Crosstalk in Antifungal Defense

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Plasmacytoid DCs (pDCs) are typically thought to be key in antiviral defense. In this issue of *Cell Host & Microbe*, Guo, Kasahara et al. (2020) reveal a critical role for pDCs in antifungal immunity. *Aspergillus*-infected monocyte-derived DCs and neutrophils recruit pDCs, which promote neutrophil fungicidal activity.

Aspergillus fumigatus is an opportunistic fungal pathogen found ubiquitously throughout the environment. Humans constantly inhale fungal spores, termed conidia, which normally do not pose a problem to healthy individuals. However, *A. fumigatus* can cause invasive aspergillosis (IA) in immunocompromised hosts and is the most common and lethal cause of mold pneumonia. Host defense against *A. fumigatus* relies on recruitment of myeloid cells, including neutrophils (NΦs), monocytes, and monocyte-derived dendritic cells (Mo-DCs), into the lung. NADPH oxidase activity in phagocytes is critical for fungal clearance in both mice and humans, as patients with chronic granulomatous disease are highly susceptible to IA. Upon exposure to reactive oxygen species (ROS) within neutrophils, conidia undergo regulated cell death, which prevents fungal dissemination and allows for sterilizing immunity (Shlezinger et al., 2017).

During *A. fumigatus* infection, CCR2+ inflammatory monocytes (iMo) are recruited into the lung, where they serve as potent proinflammatory cells and differentiate into Mo-DCs. CCR2+ iMo and Mo-DCs are required for NΦ fungicidal activity (Espinosa et al., 2017; Espinosa et al., 2014), but how they regulate NΦ function has been unknown. One possibility is that CCR2+ iMo and Mo-DCs produce inflammatory mediators that upregulate antifungal effectors within NΦs. Alternatively, CCR2+ iMo and Mo-DCs could act through a third cell type that subsequently regulates NΦ function.

In this issue of *Cell Host & Microbe,* Guo, Kasahara, and colleagues first examined the contributions of CCR2+ iMo and Mo-DCs to the production of inflammatory mediators during *A. fumigatus* infection to distinguish between the above two possibilities (Guo et al., 2020). They observed that these cell types are required for production of the chemokines CXCL9 and CXCL10. Using CXCL9- and CXCL10-reporter mice, they found that Mo-DCs and NΦs are the predominant sources of CXCL9 and CXCL10 (Figure 1). Interestingly, fungus-engaged Mo-DCs and NΦs produced much higher levels of CXCL9 and CXCL10 than uninfected bystander cells. They implicated two distinct pathways—Dectin-1/Card9-signaling and type I/III interferon (IFN) receptor signaling—in regulating CXCL9 and CXCL10 production, respectively (Figure 1). The fungal pattern recognition receptor Dectin-1 binds to β-glucan exposed during conidia swelling, the first step of germination (Hohl et al., 2005), thus indicating that Dectin-1 recognition of β-glucan in Mo-DCs and NΦs directly regulates CXCL9 production. Deficiency in type I IFN receptor (IFNAR) or type III IFN receptor (IFNLR1) signaling led to a reduction in CXCL10, a known IFN-stimulated gene. Whether cell-intrinsic Dectin-1 or IFNAR/IFNLR1 signaling in Mo-DCs and NΦs regulates chemokine production is unclear. CCR2+ iMo is a critical source of type I IFNs, and type I IFNs are required for type III IFN production (Espinosa et al., 2017). The innate immune sensing pathways that direct production of type I and III IFNs and the cellular source of type III IFN during infection are not yet known.

CXCL9 and CXCL10 are ligands for CXCR3, and the authors found that *Cxcr3*$^{-/-}$ mice are more susceptible to *A. fumigatus.* They next sought to identify the cellular target of CXCR3 required for antifungal defense. A variety of immune cell types, including T cells, NK cells, and pDCs, express CXCR3 during *A. fumigatus* infection. Lymphocytes are dispensable for *A. fumigatus* clearance (Espinosa et al., 2014), and pDCs have been previously implicated in antifungal defense (Ramirez-Ortiz et al., 2011). Thus, the authors hypothesized that CXCR3-dependent pDC trafficking to the lung promotes antifungal immunity. They observed that pDCs are recruited into the lung during *A. fumigatus* infection and that pDCs express both CXCR3 and CCR2. They found that CCR2 regulates