CellPress
Partner Journal

Molecular Plant
Spotlight

H_2O_2 sulfenylates CHE to activate systemic salicylic acid synthesis and ignite systemic acquired resistance

Reactive oxygen species (ROS), generated within plant cells during metabolic processes or in the plant apoplast when triggered by external microorganisms, comprise a group of unstable molecules that include superoxide anions, hydrogen peroxide (H_2O_2), hydroxyl radicals, and singlet oxygen and are highly reactive with other substances (Waszczak et al., 2018). In the realm of plant biology, H_2O_2 stands out as a vital ROS, instrumental in overseeing numerous developmental processes and stress responses.

Importantly, ROS, including H_2O_2, have been shown to play a key role in systemic acquired resistance (SAR), a plant's defense response that protects the entire plant against a wide range of pathogens after a local infection (Ross, 1961; Liu et al., 2024). Nonetheless, the molecular pathways through which H_2O_2 exerts its influence in SAR remain largely enigmatic. Recently, an exciting paper published in *Science* illuminated this crucial inquiry (Cao et al., 2024).

Salicylic acid (SA), a key plant defense hormone, has been identified as a central player in SAR (Fu and Dong, 2013). In *Arabidopsis* plants, the majority of pathogen-induced SA is synthesized via the ICS1 pathway (Wildermuth et al., 2001). Researchers have identified two transcription factors that bind to the *ICS1* promoter (Zheng et al., 2015). NTM-LIKE 9 is essential for SA production and vital for stomatal immunity, while the other transcription factor, CCA1 HIKING EXPEDITION (CHE), is important for pathogen-induced SA synthesis in systemic tissues. In addition, several other transcription factors, such as SARD1 and CBP60g, have been shown to bind to the *ICS1* promoter and induce SA biosynthesis in local and systemic tissues (Fu and Dong, 2013). Previous studies have recognized azelaic acid, glycerol-3-phosphate (G-3-P), N-hydroxypipecolic acid (NHP), and dehydroabietinal as mobile signals involved in SAR (Fu and Dong, 2013; Kachroo and Kachroo, 2020). However, none of these signals have been demonstrated to initiate SA production in systemic tissues.

To uncover the connection between local pathogen infection and the production of systemic SA, Cao et al. (2024) analyzed various signals induced by SAR in a recent study. Their findings revealed a significant increase in SA levels in systemic tissues prior to the observed rise in G-3-P and NHP following a local infection with the bacterial pathogen *Pseudomonas syringae* pv. *maculicola* (*Psm*) ES4326 carrying the avirulence gene *avrRpt2*. This suggests that G-3-P and NHP are unlikely to be the agents responsible for the systemic production of SA during the initiation of SAR.

To pinpoint the direct regulator of SA biosynthesis in systemic tissues, the authors concentrated on CHE, a protein that binds to the *ICS1* promoter, thereby enhancing SA production in these tissues (Zheng et al., 2015). In their intriguing study, Cao et al. (2024) found that CHE plays a crucial role in SAR, as *che* mutants exhibit impaired SAR-associated gene expression and reduced levels of NHP and SA in systemic tissues.

Subsequent research revealed that altering the single cysteine in CHE impairs its role in SAR. To delve into how CHE triggers SAR, Cao et al. (2024) concentrated on this specific cysteine residue within the CHE protein. CHE possesses just one cysteine residue, precluding the formation of an intramolecular disulfide bond. Nevertheless, disulfide bonds might still occur between two CHE molecules or between CHE and another protein. The researchers investigated this through nonreducing SDS-PAGE and discovered no evidence of such disulfide bonds. Consequently, Cao et al. (2024) explored other potential modifications of the CHE protein by both ROS and reactive nitrogen species, including S-sulfenylation (S-OH), S-sulfinylation ($S\text{-}O_2H$), and S-nitrosylation (S-NO).

Upon encountering a localized infection, Cao et al. (2024) found that *Arabidopsis thaliana* plants exhibit sulfenylation of the singular and conserved cysteine residue within the CHE protein (CHE-SOH) in neighboring and distal tissues (Figure 1). This chemical modification enhances the binding of CHE to the promoter region of *ICS1*, leading to an increase in SA production. In contrast to systemic tissues, a higher level of H_2O_2 in local tissues induces sulfinylation of CHE ($CHE\text{-}SO_2H$), which does not improve CHE's binding to the *ICS1* promoter or increase SA production. Further study will be required to find out what mechanisms cause sulfenylation of CHE in systemic tissues to enhance its affinity for the *ICS1* promoter, while sulfinylation of CHE in local tissues does not have the same effect. It remains to be determined how the higher level of SA in local tissues is achieved upon pathogen infection. Are there any post-translational modifications of NTM-LIKE 9, SARD1, and CBP60g that regulate the binding of these transcription factors to the *ICS1* promoter?

Given that a moderately elevated level of H_2O_2 in systemic tissues enhances the binding of CHE to the *ICS1* promoter, Cao et al. (2024) measured H_2O_2 concentrations in systemic tissues following infection with either the virulent bacterial strain

Published by the Molecular Plant Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and CEMPS, CAS.

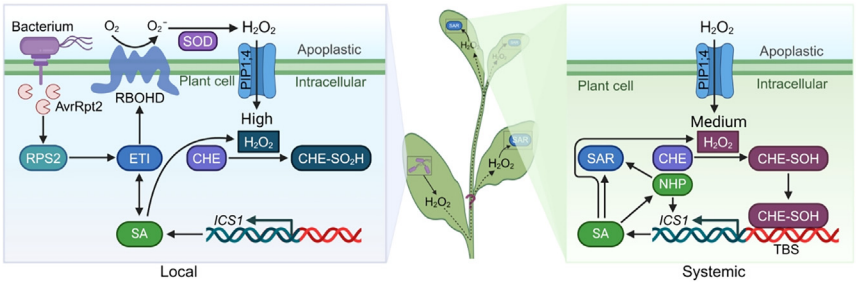

Figure 1. A schematic model illustrating H_2O_2-mediated CHE sulfenylation as a key trigger for systemic SA synthesis, leading to the activation of SAR.

Upon perception of pathogenic bacterial infection, plants initiate local defense responses, marked by the activation of RBOHD, which facilitates the production of H_2O_2, resulting in its substantial accumulation at the site of infection. This locally accumulated H_2O_2 is transported into the cell through the aquaporin channel PIP1;4, where it induces CHE sulfinylation (SO_2H). However, sulfinylated CHE lacks the ability to bind to the *ICS1* promoter, thus failing to induce SA synthesis. In addition to its local effects, H_2O_2 produced during local defense is translocated to distal leaves via an as yet undiscovered transport pathway. In these systemic tissues, medium concentrations of H_2O_2 promote CHE sulfenylation (SOH). Sulfenylated CHE exhibits an enhanced capacity to bind the TCP-binding site (TBS) of the *ICS1* promoter, thereby promoting SA synthesis and activating systemic acquired resistance throughout the plant. ETI, effector-triggered immunity. The figure was created with the software BioRender (BioRender.com).

Psm ES4326 or the avirulent strain *Psm* ES4326/avrRpt2. As anticipated, they found elevated H_2O_2 levels in local tissues compared to systemic ones (Figure 1). Additionally, treatments with SA and NHP were found to induce H_2O_2 production similar to that observed during pathogen infection. These findings align with earlier reports suggesting that SA, NHP, and H_2O_2 participate in a signal amplification loop (Cao et al., 2024).

Cao et al. (2024) employed a live-imaging technique utilizing 2′,7′-dichlorodihydrofluorescein diacetate to track H_2O_2 transport. When a half-leaf was infected with *Psm* ES4326/avrRpt2, an ROS signal was observed in the uninoculated half of the same leaf (systemic$_{nbr}$) in both wild-type and *fmo1* mutant plants, which are unable to synthesize NHP. This observation suggests that the systemic ROS signal operates independent of NHP. To assess the ROS signal at a broader level, the researchers used the ROS-responsive promoter of *GLUTAREDOXIN 13*. They discovered that, following *Psm* ES4326/avrRpt2 inoculation, ROS accumulated in systemic$_{nbr}$ tissues and in uninoculated systemic tissues 8 and 16 h post inoculation, respectively. The NADPH oxidase RBOHD utilizes NADPH to facilitate the reduction of oxygen, resulting in the generation of superoxide (Torres et al., 2002; Waszczak et al., 2018). This superoxide is then converted into H_2O_2 by the enzyme superoxide dismutase. Remarkably, the production and transport of ROS, the binding of CHE-SOH to the *ICS1* promoter, and SAR were all impaired in *rbohd* mutants (Cao et al., 2024). Intriguingly, Cao et al. (2024) discovered that, when H_2O_2 was depleted from petiole extract using catalase treatment, SAR could not be activated, even in the presence of other potential SAR inducers. However, reintroducing H_2O_2 after the catalase was removed restored the petiole extract's ability to trigger SAR, highlighting the essential role of H_2O_2 in the process. Taken together, Cao et al. (2024) have identified H_2O_2 as a groundbreaking mobile signal that initiates systemic salicylic SA production (Figure 1). Following this, SA and the mobile signals engage in a feedback loop, amplifying the signal to establish SAR.

In their research, Waszczak et al. (2014) identified approximately 100 sulfenylated cytosolic proteins in plant cells exposed to H_2O_2, indicating that sulfenylation is a widespread post-translational modification. Building on this, Hino et al. (2024) revealed that various proteins undergo sulfenylation through NADPH-mediated ROS during both pattern-triggered immunity and effector-triggered immunity. This discovery raises intriguing questions about which proteins integral to plant immunity and SAR are subject to sulfenylation, the biological consequences of these modifications, and their impact on plant defense mechanisms. Furthermore, it is essential to explore the mechanisms by which H_2O_2 is transported from local to systemic tissues and to identify any other mobile signals that might induce H_2O_2 production in systemic tissues. Beyond their roles in plant immunity, ROS, including H_2O_2, are vital in various developmental processes, such as root and leaf growth, cell proliferation, seed germination, flowering, pollen tube development, fruit maturation, and stress responses (Mittler et al., 2022; Singh et al., 2024). Intriguingly, ROS have also been recognized as crucial mediators of rapid systemic signaling for systemic acquired acclimation, a key response to abiotic stress (Gilroy et al., 2016). Although ROS are acknowledged as significant signaling molecules, their precise roles within these pathways remain to be fully elucidated. The data presented in this paper pave the way for a deeper understanding of the specific molecular functions of ROS in these crucial processes.

FUNDING

This work is supported by grants from the National Science Foundation (IOS-2207677 to Z.Q.F.) and the National Natural Science Foundation of China (32472527 to F.L.).

ACKNOWLEDGMENTS

No conflict of interest is declared.

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https://doi.org/10.1016/j.molp.2024.10.007

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