The Emergence of a Mobile Signal for Systemic Acquired Resistance

Systemic acquired resistance (SAR) is a well-known phenomenon in higher plants in which local activation of a defense response by primary infection triggers broad-spectrum resistance against microbial pathogens in the distal parts of the plant. Since its initial discovery in the 1960s, it has attracted attentions not only from scientists due to its fascinating biology, but also from agroindustry because of its potential application in crop protection. One intriguing outstanding question has been how the long distance communication is achieved in SAR.

Arabidopsis thaliana was adopted as a model organism to study the mechanism of SAR in the late 1980s. Forward genetic screens of different flavors were designed by various groups to study SAR. Studies on Arabidopsis mutants blocking the perception or biosynthesis of the defense hormone salicylic acid (SA) suggest that SA plays an essential role in SAR. However, grafting experiments using transgenic tobacco plants expressing the bacterial salicylate hydroxylase NahG indicated that SA is unlikely a mobile signal for SAR.

In addition to genes involved in SA signaling, AGD2-LIKE DEFENSE RESPONSE PROTEIN 1 (ALD1) was found to play an essential role in SAR (Song et al., 2004). In ald1 knockout mutant plants, SAR is completely lost. ALD1 encodes an aminotransferase, suggesting that it is involved in the generation of an amino acid-derived defense signal. However, its natural substrate and product was unknown for many years and how it contributes to SAR was unclear.

In 2012, the Zeier group reported in The Plant Cell that ald1 plants are deficient in the biosynthesis of pimelic acid (Pip) (Navarova et al., 2012), a non-proteinogenic amino acid known to exist in plants for decades. Upon pathogen infection, Pip accumulates in both local and systemic tissue. Treatment of Pip induces SAR-like defense priming and restores SAR in ald1 plants, suggesting that Pip is either an intermediate substrate for the production of the active signal, or itself an SAR signaling molecule. This seminal work stimulated further investigations into the biosynthesis of Pip and the mechanism of how Pip contributes to immune signaling.

Meanwhile, in a forward genetic screen to systematically isolate SAR-deficient (SARD) mutants, we obtained a large number of Arabidopsis mutants with strong SAR-deficiency phenotypes, including multiple alleles of ald1 and sard4. When SARD4 was isolated by positional cloning, it was found to encode a protein with some similarity to bacterial ornithine cyclodeaminase (Ding et al., 2016), but its exact biochemical function was unknown. In close collaboration with Dr. Ivo Feussner’s group, we found that sard4 mutant plants are deficient in Pip production. Further biochemical and metabolite analysis showed that Pip is produced using lysine as a substrate in two steps of enzymatic reactions (Ding et al., 2016; Hartmann & Zeier, 2019). ALD1 deaminates Lys to form Δ1-piperideine-2-carboxylic acid (P2C), which is further converted to Pip by the reductase SARD4. Characterization of sard4 mutants revealed

Figure 1. Biosynthesis and regulation of NHP.
A) NHP is generated from Lys through three step-wise reactions catalyzed by ALD1, SARD4 and FMO1.
B) Pathogen infection induces the expression of SARD1 and CBP60g, which in turn activates the expression of genes involved in SA biosynthesis such as ICS1, EDS5 and PBS3 as well as the expression of ALD1, SARD4 and FMO1, leading to increased SA and NHP levels. NHP further amplifies defense responses by inducing SA biosynthesis genes by a yet-to-be-determined mechanism.
that Pip still accumulates at high levels in local leaves, but is at very low levels in systemic tissue, suggesting that under physiological conditions there is very little movement of Pip from the local infected leaves to the distal parts of the plants and Pip may not serve as an SAR mobile signal (Ding et al., 2016).

Another enigmatic enzyme found to be required for SAR is FLAVIN-DEPENDENT MONOOXYGENASE 1 (FMO1). Loss of function fmo1 mutants isolated from both forward and reverse genetic studies showed strong SAR-deficiency, whereas overexpression of FMO1 leads to enhanced resistance to pathogens (Hartmann & Zeier, 2019). In fmo1 mutant plants, Pip levels are significantly higher than in wild type (Navarova et al., 2012). On the other hand, blocking Pip biosynthesis by mutations in ALD1 or SARD4 results in suppression of the constitutive defense responses in transgenic plants overexpressing FMO1 (Ding et al., 2016), suggesting that ALD1, SARD4 and FMO1 might be functioning in the same metabolic synthesis pathway, and Pip could be the substrate of FMO1.

Based on the hypothesis that FMO1 catalyzes the conversion of Pip to its N-oxidized derivative, Zeier's group performed comparative gas chromatography mass spectrometry (GC-MS) analysis on metabolites extracted from wild type, ald1 and fmo1 plants, which led to the identification of N-hydroxypipecolic acid (NHP) as a pathogen-induced metabolite that is produced in an ALD1 and FMO1-dependent manner (Hartmann et al., 2018). Subsequent in vitro assay using purified recombinant FMO1 protein confirmed that FMO1 indeed serves as a monooxygenase to convert Pip to NHP. Meanwhile, an independent study using untargeted metabolic analysis of Arabidopsis fmo1 seedlings identified O-glycosylated form of NHP, N-OGlc-Pip, as an FMO1-dependent metabolite (Chen et al., 2018). The conversion of Pip to NHP by FMO1 was confirmed using transiently expressed FMO1 protein in Nicotiana benthamiana leaves. Pre-treatment of NHP leads to enhanced resistance against virulent pathogens Psm ES4326 and Hyaloperonospora arabidopsidis Noco2 and rescues the SAR defects in ald1 and fmo1 mutants, suggesting that NHP functions as an SAR inducer.

Infiltration of local leaves with NHP induces the accumulation of N-OGlc-Pip in both local and systemic leaves, suggesting that N-OGlc-Pip can move from local to systemic tissue (Chen et al., 2018). Intriguingly, free NHP was not detectable in these samples as well as the samples pre-treated with bacteria, suggesting that either N-OGlc-Pip or another yet-to-be identified NHP-derived metabolite may serve as an SAR mobile signal. It is also possible that NHP is the mobile signal that can be rapidly converted to N-OGlc-Pip and the free NHP level is simply too low to be detected by the LC-MS method applied.

The identification of the NHP biosynthesis pathway further paved the way for studying the regulation of Pip and NHP biosynthesis (Hartmann & Zeier, 2019). SARD1 and Calmodulin-Binding Protein 60-like g (CBP60g) are two plant-specific transcription factors originally shown to regulate the induction of SA biosynthesis upon pathogen infection. Interestingly, they also promote Pip biosynthesis by activating the expression of ALD1 and SARD4. The biosynthesis of NHP is most likely regulated by SARD1 and CBP60g as well, since FMO1 was identified as a direct target of these two transcription factors in chromatin immunoprecipitation analysis, and the induction of FMO1 by Psm ES4326 is largely blocked in the sard1 cbp60g double mutant.

The identification of Pip and NHP as plant immunity inducing metabolites is a milestone in our understanding of SAR signaling. These studies also raise some important questions on the mode of action of NHP. Whether it is NHP or its derivative that is directly perceived in distal tissue remains to be determined. If NHP is the final active mobile molecule for SAR induction, how does it travel from local to distal tissues? Is there a receptor to transduce this mobile signal and how its perception leads to up-regulation of genes involved in SA biosynthesis and increased SA level? Addressing these questions will be crucial for further better understanding of the mechanism of SAR.

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REFERENCES


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