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Mini-Review

The interplay between MAMP and SA signaling

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There are two major modes for plant recognition of biotrophic microbial pathogens. In one mode, plant pattern recognition receptors (PRRs) recognize microbe associated molecular patterns (MAMPs, also called PAMPs), which are molecules such as flg22, a fragment of bacterial flagellin. In the other mode, the products of plant resistance (*R*) genes recognize pathogen effectors or host proteins modified by effectors. Salicylic acid (SA) -mediated defense responses are an important part of *R* gene-mediated resistance. It was not clear how these two signaling mechanisms interact with each other. Recently, we reported that treatment with flg22 triggered SA accumulation in Arabidopsis leaves. Disruptions of SA signaling components strongly affected MAMP-triggered gene expression responses. Flg22-triggered resistance to *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) was partly dependent on SA signaling. Our results demonstrated the importance of SA signaling in flg22-triggered resistance and, at the same time, the importance of some other signaling mechanism(s) in this resistance. Here we discuss potential signaling components of flg22-triggered SA accumulation and other signaling mechanisms potentially contributing to flg22-triggered resistance to *Pst* DC3000.

Two Modes of Defense: PRR and *R*-Gene-Mediated Defenses

Plants are subject to attack by a wide variety of microbial pathogens. In response, plants induce numerous defense mechanisms.[1] Perception of biotrophic microbial pathogens by plants can be divided into two main modes.[2,3] In one mode, pattern recognition receptors (PRRs) sense microbe-associated molecular patterns (MAMPs), molecules such as flg22, a fragment of bacterial flagellin.[4] This triggers a signaling cascade that activates plant defense mechanisms such as cell wall reinforcement by callose deposition,[5] the production of reactive oxygen species (ROS),[3] and the induction of numerous defense-related genes.[6] In the other mode, the products of plant resistance (*R*) genes recognize pathogen effectors or host proteins modified by effectors, and trigger *R*-gene-mediated defense responses.[7] Effectors are pathogen proteins or metabolites that promote virulence, often by interfering with host defense responses.[7]

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Resistance gene-mediated defense responses are usually accompanied by a rapid production of ROS. ROS are involved in hypersensitive cell death (HR), a type of programmed cell death thought to limit the access of pathogens to water and nutrients. Resistance gene-mediated resistance is also associated with activation of a salicylic acid (SA)-mediated signaling pathway that leads to the production of many pathogenesis-related (PR) proteins which are thought to contribute to resistance.[1,8]

The response to flg22 is the best characterized MAMP response. Flg22 binds to a plant membrane-localized receptor-like kinase, FLS2,[9] triggering an oxidative burst catalyzed by the *AtrbohD*-encoded NADPH oxidase,[10] activating mitogen-activated protein kinase (MAPK) cascades,[11,12] and inhibiting growth of *Pseudomonas syringae*.[13]

The Interplay Between Mamp-Triggered and SA-Mediated Defense Responses

By studying expression profile data from Arabidopsis responding to MAMPs such as flg22 or to SA, we noticed extensive overlap between the sets of genes regulated by MAMPs and SA. This suggested that there was an interaction(s) between MAMP and SA signaling. However, the mechanisms underlying the interaction were not known.

In our recent paper,[14] we showed an intimate interaction between MAMP-triggered and SA-mediated signaling mechanisms. We found that SA accumulated after flg22 treatment. This accumulation was dependent on *SID2*, which encodes isochorismate synthase, an SA biosynthetic enzyme.[15,16] Analysis of transcriptional responses using a custom DNA microarray[17] revealed a group of genes that are induced by MAMPs in a *SID2*-dependent manner. Another group of MAMP-induced genes was *SID2*-independent at early time points but *SID2*-dependent at later time points. In wild-type plants, pretreatment with flg22 reduces growth of *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000).[13] Importantly, in SA-deficient *sid2* plants, this effect was attenuated but not abolished, demonstrating the importance of SA signaling in flg22-triggered resistance. The fact that the resistance was not abolished indicated that another signaling mechanism(s) must also contribute to resistance.

Potential Mechanisms for SA Accumulation in Response to Flg22

PAD4, *EDS1* and *EDS5* are required for activation of SA accumulation in response to some, but not all, SA-inducing stimuli.[18-20] We

showed that *PAD4* is involved in flg22-triggered SA accumulation and resistance to *Pst* DC3000. However, the signaling mechanisms that lead to SA accumulation are still largely unknown although SA-mediated defense responses have been studied extensively.[21]

Flg22 recognition triggers the activation of several MAPK signaling cascades within 10 minutes after treatment. SA accumulation was significantly induced 3 to 6 hours after flg22 treatment.[14] Therefore, MAPK cascades could be upstream signaling components for SA accumulation. MAPK3 and MAPK6 are activated by flg22, but it is not known whether they are involved in activating SA accumulation. Supporting the idea that MAPK cascades are involved, overexpression of *AtMKK7*, which encodes MAPKK7, leads to high levels of SA accumulation.[22] Furthermore, *ATMKK7* transcription is induced by flg22 treatment. However, we found that flg22-triggered induction of *PR-1*, a good marker gene for SA accumulation,[23] was not compromised in *atmkk7* antisense transgenic plants, suggesting that MAPKK7 is not required for flg22-induced SA accumulation (Kenichi Tsuda and Fumiaki Katagiri, unpublished data). MAPK4 is a negative regulator of SA accumulation since SA levels are constitutively high in *mpk4* mutants.[24] However, it is unlikely that downregulation of MPK4 leads to SA accumulation in response to flg22 since MAPK4 is activated by flg22.[12] It remains possible that MAPK cascades are involved in activating SA accumulation, but to date there is no data supporting this idea.

ROS production is induced within 10 minutes after flg22 treatment in an *AtrbohD*-dependent manner.[10] ATRBOHD is a negative regulator of *lsd1*-mediated runaway cell death, which requires SA accumulation.[25] Therefore, it is unlikely that ATRBOHD is a positive regulator of SA accumulation in response to flg22. Rather, it is likely that ATRBOHD is a negative regulator of SA accumulation. Flg22-triggered callose deposition, which is downstream of ATRBOHD,[10] could also be a negative regulator of SA accumulation since SA signaling is constitutively activated in a callose-deficient mutant.[26] Thus, the mechanism of flg22-induced SA accumulation remains unknown.

The Signaling Pathways Required for Flg22-Triggered Resistance to *Pst* Dc3000

We demonstrated that flg22-induced resistance to *Pst* DC3000 is partially dependent on SA signaling.[14] Resistance was not impaired in jasmonic acid (JA) or ethylene (ET) signaling mutants although treatment with flg22 also appears to induce these hormone signaling pathways.[13] The plant hormones JA and ET play major roles in signaling during defense responses. Loss of JA or ET signaling tends to increase susceptibility to necrotrophic pathogens, and to have little effect on resistance to biotrophs.[1] There is mutual negative cross-talk between JA and ET signaling on one side, and SA signaling on the other.[1] The biotroph *P. syringae* takes advantage of this crosstalk by producing an analog of JA-isoleucine, the active form of JA, that reduces the strength of SA signaling in host plants.[27,28] Therefore, JA and ET signaling triggered by flg22 may not be important for the resistance to *Pst* DC3000. However, there is also evidence that JA, ET and SA signaling may sometimes act synergistically.[29] Our observation demonstrated the importance of some other signaling mechanism(s) in addition to SA signaling in flg22-triggered resistance to *Pst* DC3000. One possibility is that unknown signaling mechanism(s) are involved in the resistance. Alternatively, the resistance could be mediated by known mechanisms, such as SA, JA and ET signaling, acting in a highly redundant manner. These two possibilities are not mutually exclusive. In the latter case, combining mutations that block multiple known signaling mechanisms could reveal such redundant regulatory mechanism(s).

Concluding Remarks

Our study revealed one type of interaction between MAMP-triggered and SA-mediated signaling: MAMP perception triggers SA accumulation. However, the interaction among signaling mechanisms involved in the MAMP-triggered response is clearly complex, and more research is needed to reveal all the relationships.

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