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MINI-REVIEW



Plant immune responses - from guard cells and local responses to systemic defense against bacterial pathogens

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ABSTRACT

When plants are infected by pathogens two distinct responses can occur, the early being a local response in the infected area, and later a systemic response in non-infected tissues. Closure of stomata has recently been found to be a local response to bacterial pathogens. Stomata closure is linked to both salicylic acid (SA), an essential hormone in local responses and systemic acquired resistance (SAR), and abscisic acid (ABA) a key regulator of drought and other abiotic stresses. SAR reduces the effects of later infections. In this review we discuss recent research elucidating the role of guard cells in local and systemic immune responses, guard cell interactions with abiotic and hormone signals, as well as putative functions and interactions between long-distance SAR signals.

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Introduction

Phytopathogens cause numerous diseases that can lead to decreases in crop yield, economic losses, and they threaten global food security and natural resources.¹ Because plants do not have the mobile immune cells that vertebrates have, the plant immune system has been thought to be far less complex and has been less studied. However, plants are capable of highly-specific immune responses.^{2–4} In addition, recent studies suggest that plants can generate a lifelong “memory” of pathogen interaction, referred to as “priming”, and that they pass the immunity to their offspring through epigenetic changes. In the “primed” state, plants maintain an enhanced ability to activate cellular defense responses quickly and effectively.^{5,6}

The particular plant–pathogen interaction that is the focus of this review is between *Arabidopsis thaliana* (a reference plant with rich genetic resources) and the bacterium *Pseudomonas syringae*. During infection, the pathogen secretes coronatine, a molecule that mimics the plant hormone jasmonate-isoleucine and enables bypass of plant immune defenses.^{7–10} Stomata, which are pores formed by pairs of guard cells on the surfaces of leaves that function in gas exchange and transpiration, respond to pathogen infection^{11–13} as well as environmental cues including light, carbon dioxide, and humidity.^{14–18}

Although there are some general aspects to plant immunity that are well characterized, plant–pathogen responses vary greatly from one species to another, and there are still a lot of gaps in our knowledge. For example, local responses to certain pathogens have been well characterized, but there is still a need for information in long distance signaling, how the signals confer immunity, how the immunized tissues respond, and

how this is passed on to future generations.^{19–21} Additionally, recent reviews of plant immunity have not focused on the important role that guard cells play in the initial localized response to pathogen attack, and research on the systemic response that confers immunity has not included the potential role of guard cells.^{2,4} Recent research found that the plant hormone salicylic acid (SA), which regulates immune responses in systemic acquired resistance (SAR), can also regulate stomatal closure through the cell-specific transcription factor NTM1-LIKE 9 (NTL9).^{22,23} Therefore, in addition to serving as a primary defense against local pathogen attack, primed guard cells in systemic tissues may play additional roles in preventing future infections as a part of the overall plant immunity.

Local responses to pathogens: PAMP, PTI, and ETI

The site where the pathogen enters a plant organ is referred to as the primary infection site, and the molecular responses in the infected tissues are local responses. Phytopathogens encounter many obstacles when attacking a plant cell, the first being the plant cell wall. Upon activation of defense pathways, the cell wall is further reinforced by deposition of callose (a glucan polymer) at the site of pathogen attack. The plant’s first line of the defense against the invading pathogens are receptors localized in the plant cell membrane that recognize pathogen-associated molecular patterns (PAMPs) or microorganism-associated molecular patterns (MAMPs). These PAMPs (or MAMPs) are conserved, microbial-specific molecules from the pathogen and include lipopolysaccharides, flagellin, and chitin. One type of pattern recognition receptor (PRR) has a conserved structure consisting of an extracellular leucine-rich repeat (LRR) domain and an

intracellular protein kinase domain. Several PRRs have been characterized (Figure 1) and include transmembrane EF-Tu receptor (EFR) and Flagellin Sensitive 2 (FLS2). FLS2 is analogous in structure to human Toll-like receptor 5 (TLR5), and it recognizes the pathogen elicitor flg22, a peptide corresponding to the conserved domain of flagellin2 *A. thaliana* also has receptor-like kinases (RLKs) and receptor-like cytoplasmic kinases (RLCKs) that mediate transmembrane signal transduction as part of plant immunity.²⁵ One such RLK, brassinosteroid insensitive 1 (BRI1)-associated receptor kinase 1 (BAK1) associates with FLS2 which associates with BOTRYTIS-INDUCED KINASE1 (BIK1), a RLCK that functions downstream of BAK1 to activate the respiratory burst oxidase homolog D (RBOH D) and trigger reactive oxygen species (ROS) burst.²⁵ This initial recognition of pathogen attack together with the basal defense response triggered by the PRRs is referred to as PAMP triggered immunity (PTI). PTI is characterized by several molecular and physiological changes in the infected cell including alkalinization, changes in ion flux, increase in ROS and nitric oxide (NO), and activation of mitogen-activated protein kinase (MAPK) cascades.^{26–29} The overall effect of PTI is to curtail pathogen growth.

Certain pathogens, including bacterial pathogens with type-III secretion pili and some fungal pathogens, have

developed ways to suppress PTI. Pathogenic bacteria in the genera of *Pseudomonas*, *Ralstonia*, *Xanthomonas*, and *Erwinia* can suppress the primary PTI defense of the plant via injection of strain-specific effector molecules into the plant cell.^{30–32} However, plants have a second level of immune response, called effector-triggered immunity or ETI, which developed through co-evolution with specific pathogens.³³ When effectors are recognized by intracellular nucleotide-binding (NB)-LRR receptors, ETI is triggered. As part of ETI, plants have intracellular receptors known as resistance (R) proteins that detect the presence of specific effector proteins known as avirulence (Avr) or type-three effectors (T3E). When Avr proteins are recognized by the plant's R proteins, a type of localized programmed cell death (PCD) called hypersensitive responses (HR) results in restriction of the pathogen spread. Additionally, interactions of Avr effectors with R proteins lead to the expression of pathogenesis related (PR) genes, which are tightly linked to SAR³³ that increases the resistance of distal tissues to infection. The beneficial effects of SAR³⁴ are enhanced resistance to a broad spectrum of pathogens in non-infected parts of the plant.⁴ However, the HR and local senescence are not required for SAR, as evidenced by recent research with *P. syringae maculicola*.³⁵ Acronyms related to plant immune response are summarized in Table 1.

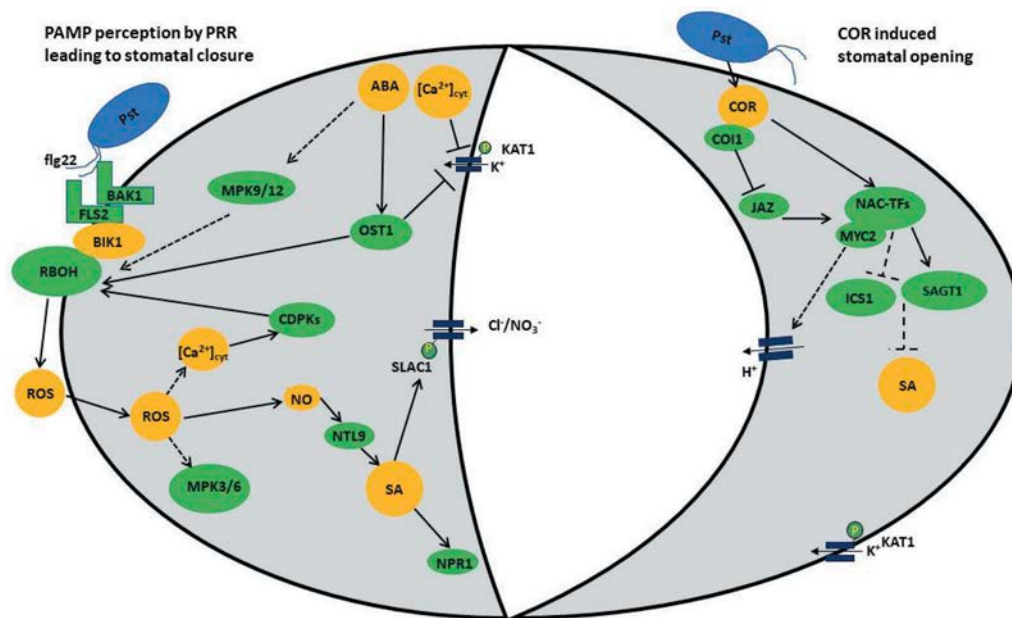


Figure 1. Stomatal guard cell response to *Pseudomonas syringae*. Guard cells play an early role in plant immunity and respond to the pathogen *P. syringae* in a number of ways. Aperture of the stomatal pore is modulated by turgor pressure of the surrounding guard cells, and the solute potential is controlled by anion channels and K^+ channels. During stomatal closure, these channels are regulated by a series of signaling events initiated by perception of the pathogen by the guard cell. PAMPs (or MAMPs), like flg22, released from the pathogen are recognized by transmembrane receptors such as FLS2 and BAK1. BAK1 associates FLS2 which in turn associates with BIK1 to activate RBOH, and initiate ROS burst. MPK3 and MPK6, as well as RBOH, NO, and ROS burst are downstream effects of FLS2 recognition of flg22. PAMP perception also triggers Ca^{2+} channels leading to intercellular Ca^{2+} increase and CDPK activation. The overall effect of PTI and ETI is to stop the spread of the pathogen. While generation of ROS signals play an important role, various plant hormones including SA, JA, and ABA also play a role in this stomatal response. Some evidence shows that SA-induced stomatal closure is linked to NO and the NTL9 transcription factor which controls SA biosynthesis in this process. Increase in SA activates NPR1, a key regulator of SAR signaling. Increased levels of endogenous ABA during the biotic stress inhibit H^+ extrusion, activate Ca^{2+} uptake channels, and also activate both slow 'S-type' and rapid 'R-type' anions efflux channels. These changes lead to K^+ efflux via the voltage-gated outward K^+ channel GORK and membrane depolarization.²⁴ These K^+ and anion changes increase water potential in the guard cells and drive water to move out, causing stomatal closure. ABA also activates stomatal closure via the activation of the serine-threonine protein kinase OST1. Phosphorylation by OST1 activates SLAC1 anion channels and inhibits the KAT1 K^+ inward rectifying channels. Ions move out and water potential in the guard cells is increased, water moves out and turgor pressure decreases, resulting in closure of the stomatal pore. During pathogen-induced stomatal opening, the pathogen effector COR structurally mimics the plant hormone JA-Ile and binds to COI1 to trigger the degradation of JAZ proteins. Additionally, COR induces NAC transcription factors that bind MYC2 and repress SA synthesis leading to reopening of stomata.

Table 1. Acronyms and abbreviations related to plant immune response. Acronyms and abbreviations for factors involved in plant immunity that are mentioned in this review are listed with their meanings and a brief summary of their functions. These factors include both local and systemic plant immune responses.

Acronym/ Abbreviations	Full name	Function
PAMP/MAMP	Pathogen- or microbe-associated molecular patterns	Microbial molecules recognized at plant cell surface
PRRs	Pattern recognition receptors	Recognizing PAMPs and activating PTI
PTI	Pattern (or PRR)-triggered immunity	Activation of plant defense following PAMP recognition
AvrPto, flg22, HopA11	Effectors	Proteins or other molecules produced by pathogens to interfere with and suppress plant defense mechanisms or their activation
ETI	Effector-triggered immunity	Secondary plant defense, usually stronger than PTI
R genes or proteins	Resistance genes or proteins	Mediating specific resistance and triggering ETI
CDPKs	Calcium-dependent protein kinases	Induction of cytoplasmic signaling cascades by phosphorylation of substrates
MAPKs	Mitogen-activated protein kinases	Induction of cytoplasmic signaling cascades by phosphorylation of substrates
NADPH oxidases	Nicotinamide adenine dinucleotide phosphate-oxidases	Generating ROS
ROS	Reactive oxygen species	Signaling molecules, antimicrobial properties, leading to cell death
LRR	Leucine rich repeat	Ubiquitous motif found in R proteins
TF	Transcription factors	Proteins that control rate of transcription
SAR	Systemic acquired resistance	Activated in plant tissue distant to initial infection, leading to reduction in disease symptoms
PR genes	Pathogenesis-related genes	Regulated by SA, accumulating minutes to hours after PTI or ETI
SA	Salicylic acid	Required for signal perception and SAR response in distal tissues

Role of guard cells in perception of pathogen attack

Guard cells are dynamic specialized cells that respond to many different environmental cues, allowing them to close the stomata in unfavorable conditions and then open the stomata when the environment is favorable. Guard cells have the unique ability to integrate multiple, diverse, and at times conflicting signals in response to light, photoperiod, abiotic stress, and biotic stress and translate them into appropriate physiological and physical responses.^{14,16,17,36}

Although it was previously assumed that these surface openings were merely passive portals of entry for foliar plant pathogens, recent research has shown that guard cells are pathogen responsive and play an early role in the plant immune response.^{7,11,12} Guard cell perception of PAMPs leads to a cell-specific response, and stomata closure is triggered. When leaves or epidermal peels of *A. thaliana* were exposed to a suspension of *P. syringae* pv tomato (*Pst*) DC3000, a marked reduction in the number of open stomata (from circa 70–80% to 30%) was observed within 1–2 h of incubation, and the average stomatal aperture was reduced from approximately 2 μm to 1 μm .¹¹ Perception of PAMPs by plant cell PRRs triggers

calcium channel activation in guard cells, increasing Ca^{2+} which then activates calcium-dependent protein kinases (CDPK). CDPK then activates a plasma membrane localized NADPH oxidases (RBOHs) and induces extracellular ROS production. CDPK also activates anion channels leading to membrane depolarization, which in turn activates outwardly-rectifying K^+ channels and K^+ efflux. This decreases guard cell turgor and leads to stomatal closure. Stomatal closure is also promoted by the phytohormones SA, jasmonic acid (JA), and abscisic acid (ABA). Interestingly, these phytohormones also regulate pathogen responses in SAR, which will be discussed later. Playing a central role as a common regulator of many of these guard cell signal transduction pathways is OPEN STOMATA 1 (OST1, aka SnRK 2.6), a serine-threonine protein kinase that initiates downstream signaling.^{37,38} OST1 was identified by Mustilli et al. using infrared thermal imaging to screen a population of ethyl methane sulfonate-mutagenized *Arabidopsis* for mutants with lower leaf temperatures.³⁸ Mutations in OST1 prevent stomatal closure in the presence of ABA.³⁸ OST1 phosphorylates a number of substrates including the RBOH and slow anion channel-associated 1 (SLAC1) S-type anion channel, which is activated by OST1. Another substrate, K^+ inward rectifying channels in *A. thaliana* (KAT1) are inhibited by OST1 phosphorylation.³⁹ The promotion of anion efflux and suppression of cation influx promote decrease in turgor pressure in guard cells and stomatal closure. OST1 also targets RBOH that generates ROS. Bursts of ROS activate Ca^{2+} channels in both the plasma membrane and vacuolar membrane, and the resulting changes in Ca^{2+} in the cytosol lead to downstream signaling responses.³⁹

When stomata in leaves or epidermal peels of *A. thaliana* are exposed to a suspension of *Pst* DC3000, they close within 1–2 h and reverted to an open state after 3 h.¹¹ As mentioned earlier, the opening is due to the effector molecule produced by some strains of *P. syringae* called coronatine (COR), which structurally mimics JA-isoleucine (JA-Ile), the active form of the hormone,¹⁸ and induces stomatal opening as shown in Figure 1. COR binds to CORONATINE INSENSITIVE 1 (COI1), an F-box protein that senses JA-Ile. This binding triggers COI1-dependent degradation of the substrates of COI1, the JASMONATE ZIM DOMAIN (JAZ) proteins.⁴⁰ COR also induces expression of three transcription factors (TFs) in the NAC family, *ANAC019* (At1G52890), *ANAC055* (At3G15500), and *ANAC072* (At4G27410), which have binding sites for the MYC2. MYC2 is a TF released from a complex with JAZ, after COR-triggered degradation of JAZ proteins. A triple knockout mutant of the NAC TFs is insensitive to COR-triggered stomatal reopening and maintains higher levels of SA than wild type *Arabidopsis* after bacterial inoculation. The NAC TFs putatively decrease SA synthesis via transcriptional repression of isochlorogenic acid synthase gene 1 (ICS1) and increase SA storage via SA glucosyl transferase gene 1 (SAGT1), aiding in the reopening of stomata.^{18,41,42}

JA and SA often exhibit antagonistic effects in plant defense responses.^{43,44} *P. syringae*-triggered stomatal closure in both *Phaseolus vulgaris* and *A. thaliana* was found to be

compromised during high relative humidity, which was accompanied by early up-regulation of the JA pathway and simultaneous down-regulation of the SA pathway in guard cells.⁴⁵ Recent results show that a transcription factor NTM1-LIKE 9 (NTL9) is required for SA biosynthesis in guard cells and closure of stomata in response to pathogens, linking increase of SA to the stomatal closure response.²² Additionally, results from two *Arabidopsis* NO mutants, *nial* and *nial2*, demonstrate that SA-induced stomatal closure is regulated by NO generation in guard cells.²³

Convergence of biotic and abiotic stress signaling

While guard cells are able to perceive pathogen attack and close stomata, they also respond to abiotic conditions including heat, drought, high or low CO₂, and high or low humidity. ABA is a key regulator that controls many abiotic stress responses, including stomatal movement.⁴⁶ In guard cells, levels of endogenous ABA increase during abiotic stress and cause stomatal closure by decreasing solute potential.²⁴ However, in other plant cell types during combined abiotic and biotic stress response, ABA can act either synergistically with JA or antagonistically with SA during stress responses.⁴⁶ Exogenously applied or endogenously produced ABA is correlated with increased bacterial growth in *Arabidopsis* challenged with *P. syringae*, and the ABA biosynthetic mutant *aba3-1* showed reduced susceptibility to *P. syringae*.⁴⁷ Overall, ABA may act to fine tune the cellular response to pathogens by promoting JA signaling while suppressing SA signaling.⁴⁸ This hormone crosstalk is specific for the type of pathogen, in that biotrophic pathogens induce SA biosynthesis and SAR, while necrotrophic pathogens induce ethylene and JA biosynthesis and signaling that are antagonistic to SA.⁴⁶

Several MPKs are activated to induce stomatal closure in response to abiotic and biotic stresses. Both MPK3 and MPK6 function interdependently of ABA signaling, and both the ABA and MPK3/6 cascades are required for stomatal immunity.^{39,49} The MPK3/6 cascade leads to decrease in malate/citrate levels in the guard cells and altered osmolarity to reduce turgor pressure and close the stomata, whereas ABA signaling activates OST1, which phosphorylates RBOHD and SLAC1.^{39,49} Cl⁻ efflux mediated by SLAC1 leads to K⁺ efflux from guard cells and stomatal closure. MPK9 and MPK12 are guard-cell specific and involved in ABA and ROS responsive stomatal closure via SLAC1.⁴⁹

Convergence of these biotic and abiotic signaling pathways has been the subject of investigation for decades. The complex interplay between the susceptibility of the plant, the inoculum potential of the pathogen, and the impact of the environmental conditions is a concept first described in the 1960s by George McNew as the disease triangle.⁵⁰ The disease triangle is a concept regarding the role of the environment in disease control. McNew proposed that there were six factors relating the environment, pathogen, and plant, and they interact to determine the impact of pathogens on a host under favorable conditions. These factors include: the physical environment (temperature, humidity, etc.) and duration of the infection, pathogen prevalence and virulence, age of the plant, and

inherent susceptibility of host plant. The disease triangle concept is widely accepted in the field of plant pathology and is supported by current research.^{50,51} Recent transcriptomic analysis found 138 pathogen-induced genes that exhibited stronger transcriptional response when the host plant *Vitis vinifera* was exposed simultaneously to infection by the pathogen *Xylella fastidiosa* and drought stress than either stress alone.⁵² These molecular results correlated to the physiological and phenotypic results with respect to water content, photosynthesis rate, severity of disease symptoms, and extent of pathogen colonization. Similarly, results of microarray and RT-qPCR experiments showed 20 novel genes that were uniquely regulated during concurrent drought and pathogen stress in *A. thaliana* infected with *Pst* DC3000.⁵² Future studies involving other abiotic and biotic stress combinations will greatly facilitate elucidation of the “cross-tolerance” mechanism in the disease triangle.^{54–56}

Mobile signals for SAR

For SAR to occur, mobile signals must be generated at the site of infection and move to uninfected tissues. Most of these signals are thought to travel via the plant vasculature (xylem and phloem), however, there is some evidence for volatile signals as well.^{21,57} There are six metabolites that have been identified as potential long-distance signals, which include methyl salicylate (MeSA), glycerol-3-phosphate (G3P), DEFECTIVE IN INDUCED RESISTANCE 1-1 (DIR1), azelaic acid (AzA), dehydroabietinal (DA), and pipecolic acid (PiP). Evidence for their being mobile signals is reviewed by Dempsey and Klessig (2012).²¹

Methyl salicylate (MeSA)

MeSA increases in leaves inoculated with pathogens that induce SAR response.²⁰ When SA accumulates in pathogen-infected leaves, some of it is converted to MeSA. As MeSA accumulates, it travels to systemic leaves via the phloem. Once in the systemic leaves, MeSA is converted back to SA. This newly released SA triggers (primes) systemic defense responses, as will be discussed in the next section.⁵⁸ MeSA has also been implicated as an airborne signal for plant defenses.⁵⁹

Glycerol-3-phosphate (G3P)

SUPPRESSOR OF FATTY ACID DESATURASE DEFICIENCY 1 (*SFD1*) encodes a glycerol-3-phosphate dehydrogenase (G3PDH), aka dihydroxyacetone phosphate (DHAP) reductase.^{60,61} This enzyme is required to catalyze the reduction of DHAP in an NADH-dependent manner to generate G3P. This is a necessary precursor for the synthesis of all glycerolipids, which include membrane and storage lipids. Activity of G3PDH expressed from *SFD1* is required in the chloroplasts for SAR and lipid metabolism to occur.²¹ The signal that is derived from *SFD1* may be G3P, but this is still unclear. G3P increases in leaves inoculated with SAR-inducing pathogens and azelaic acid (AzA) promotes

accumulation of G3P. In addition, G3P also promotes the conversion of MeSA to SA in distal leaves.²⁰

Defective in induced resistance 1-1 (*DIR1*)

DIR1 encodes a novel type of lipid transfer protein (LTP) that is able to bind two molecules of long-chain fatty acids with high affinity.^{62,63} Evidence suggests that *DIR1* can enter the phloem via the cytosol of companion cells and act as a long-distance chaperone to systemic tissue for three other long distance SAR signaling molecules, G3P, dehydroabietinal (DA), and AzA.^{4,64} *DIR1* appears to interact with itself and AZELAIC ACID-INDUCED 1 (*AZI1*) (see below). Both *DIR1* and *AZI1* are required for G3P- and AzA-induced SAR. The presence of *DIR1* and *AZI1* enhance sensitivity to the signaling form of DA (see below).²⁰

AzA

Treatment with AzA did not induce SA accumulation or PR expression, but did enhance PR gene activation after secondary pathogen inoculation. *AZI1* is a predicted secreted protease inhibitor/seed storage protein/LTP, which is not homologous to *DIR1*. *AZI1* is possibly involved in the production or transport of the mobile SAR signal, but not the perception of it.^{65,66} AzA levels increase in leaves inoculated with SAR-inducing pathogens and AzA is transported to distal leaves.²⁰

DA

Both DA and AzA were found in an analysis of small molecules enriched in the petiole exudates of Arabidopsis plants treated with avirulent (Avr) *P. syringae*. AzA and DA can induce local and systemic resistance to virulent pathogens when applied locally. However, DA appears to require the interaction of one or more proteins for SAR induction, as trypsin treatment was able to abolish the SAR-inducing activity.^{67–69} During SAR, total content of DA does not change and it is proposed that DA is mobilized from a non-signaling form to a signaling form which is presumably transported via the phloem.²⁰

Pipecolic acid (*pip*)

PiP is a product of lysine degradation,²¹ found in elevated levels in petiole extracts and systemic leaves of pathogen-inoculated plants. Like AzA, PiP does not increase SA levels, but primes systemic tissue for faster and stronger secondary response.^{70,71} PiP is induced by *P. syringae* in an AGD2-LIKE DEFENSE RESPONSE PROTEIN1 (*ALD1*)-dependent manner^{70,73} and is suggested to amplify its own synthesis as well as the activity of the SA via an SA synthesis enzyme (ICSI) possibly by a transcriptional amplification loop.^{20,70} SA amplification involves FMO1 (FLAVIN-DEPENDENT MONOOXYGENASE 1) and PAD4 (PHYTOALEXIN-DEFICIENT 4). The SA amplification loop activates NON-

EXPRESSER OF PR1 (*NPR1*) by SA, ultimately resulting in SAR signaling.²⁰

Research on mobile signals that induce SAR has focused on the transmission of these signals from infected mesophyll cells to systemic non-infected mesophyll cells, but has not addressed potential interactions with guard cells, which have been shown to respond to pathogen infection.¹¹ Signals from mesophyll apoplasts can induce stomatal responses to CO₂ in *Commelina communis*.⁷⁴ Furthermore, by using mesophyll segments that were sandwiched by gel and doughnut-shaped spacers made of either cellophane or polyethylene, the authors found that the mesophyll signals that move to the epidermis were in an aqueous, not a gaseous phase.⁷⁴ Interestingly, they also found that stomatal opening was dependent on mesophyll photosynthesis, but that stomatal closure was less dependent on mesophyll photosynthesis signals. This demonstrates that guard cells can perhaps receive signals both from the mesophyll cells and also from the outside environment to induce stomatal movement. As guard cells mature, their plasmodesmata become truncated and nonfunctional, which eliminates intercellular communication with surrounding epidermal cells.⁷⁵ However, plants maintain some plasmodesmata between the bundle sheath/phloem parenchyma cells and the sieve element-companion cell complexes possibly for of apoplastic loading and long distance intercellular communication.⁷⁶ It seems plausible that plants cells would require a method of exchange of mobile signals during pathogen attack, and that different cell types would be involved in this signaling process.

Signal amplification in systemic leaves

When long-distance SAR signaling molecules reach distal tissues from pathogen infected tissues, they must be perceived by the cells in the systemic tissues in order to initiate the global SAR response. Although the phenomenon of SAR has been known since the 1930s, the receptors of mobile signals (see the previous section) have not been identified. What is best known is that SA is accumulated in the systemic tissue sometime after the mobile signal is sent. SA accumulation leads to the secretion of antimicrobial pathogenesis-related (PR) proteins. This leads to the rest of the plant being protected from a broad spectrum of pathogens for weeks to months. PR proteins and peptides consisting of a large number of members which differ in occurrence (spatial and temporal), expression levels, and biological activities.⁷⁷

The master regulator of SA signaling is *NPR1*.⁷⁸ *NPR1* was first identified in *A. thaliana* and is required for PR gene expression, SA signaling and SAR.⁴ SA directly binds to *NPR1* adapter proteins. *NPR1* is one of ten TGA transcription factors (a family of transcription factors with a TGACG motif) in Arabidopsis, and seven of them have been found to interact with *NPR1* in yeast two-hybrid screens.⁴ TGAs have been shown to bind directly to PR gene promoters. PR proteins are the activators of SAR with a molecular weight range of 5–75 kDa that are secreted or targeted to the vacuole.⁷⁷ It is difficult to test the

contribution of each PR protein because they work in concert and are encoded by multiple genes in gene clusters.⁷⁷ One point of confusion in the field is the temporal aspect of the SAR response, as treatments are done in different plants species, at different ages, in different organs or leaves, and under different growth conditions. In general, expression of PR genes is up-regulated within 24–48 h in systemic tissues after the primary inoculation.⁴

Kumar et al. found that inoculation of Arabidopsis plants with the rhizobacteria *Bacillus subtilis* FB17 via the roots, restricted the entry of *Pst* DC3000 through the stomata when the plants were co-inoculated with both microorganisms.⁷⁹ Arabidopsis plants that were inoculated only with the rhizobacteria showed reduced stomatal aperture one hour after inoculation, similar to inoculation with *Pst* DC3000. However, the rhizobacteria-inoculated plants maintained a reduced stomatal aperture even at three hours post-inoculation, unlike *Pseudomonas*-inoculated stomata that reopen after three hours.⁷⁹ This demonstrates the interconnection between environmental conditions and immune response, and the ability of the plant immune system to prepare systemic tissues for response to different microbial organisms.

Defense priming in systemic tissues

A relatively new and exciting aspect of SAR research is the SAR defense priming or conditioning. This can be thought

of as enabling the plant to maintain a vigilant or alarmed status in which they are able to react faster and more effectively to pathogen attack.⁸⁰ Research by Jung et al. has shown that exogenous AzA treatment enabled plants to accumulate higher levels of SA and *PR1* transcripts.⁵ They also found that primary inoculation with SAR-inducing pathogens led to defense priming in distal leaves, and that the whole plant mobilized defense more rapidly upon secondary infection. Exogenous application of PiP had similar effects, in that primed plants had stronger induction of defense genes after an initial challenge infection.⁷⁰ Priming is found to be dependent on two MAP kinases, MPK3 and MPK6.⁸¹ These SAR signaling mechanisms and priming responses are summarized in Figure 2.

One issue with the research on priming is that it is largely based on gene expression data of whole leaf tissue. This masks the responses of specialized cells such as guard cells and it overlooks protein and metabolite regulations (e.g., post-translational modifications and hormone cross-talk) that may play important roles in the priming and the strong response of the primed tissue. Future research focusing on single cell types and their cross-talk in local and distant tissues will greatly improve knowledge of plant defense mechanisms, which can be applied toward enhancing plant productivity and food security.

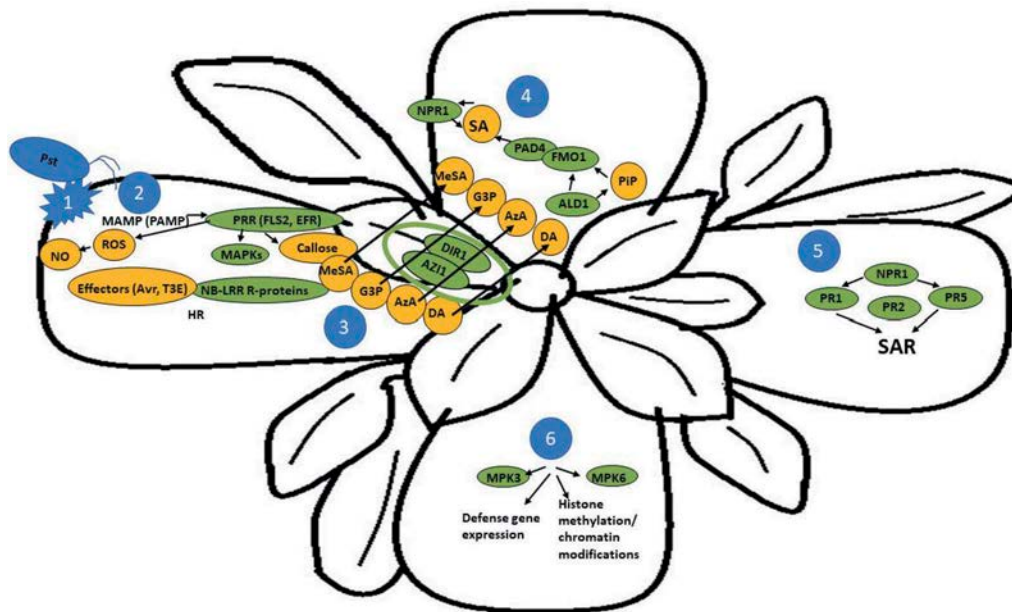


Figure 2. Local and systemic response to *P. syringae* pv. Tomato (*Pst*) infection in *A. thaliana*. Plant immune response requires the convergence and interaction of both localized pathogen response pathways as well as initiation of enhanced pathogen resistance in non-infected systemic tissues. The process begins with 1) the primary infection by *Pst*, which then triggers 2) PTI through perception of MAMP (PAMP) by PRRs and the activation of MAPKs and production of NO, ROS, and callose deposition. Additionally, certain pathogens including *Pst* activate ETI response by producing effector proteins that bind to NB-LRR containing R proteins in the plant. This often, but not always, triggers HR response in the locally infected plant tissue. 3) Putative SAR signals including DIR1, G3P, DA, AzA, and MeSA are produced in the infected tissue and transported to systemic tissues. DIR1, a novel type of lipid transfer protein, acts as a long-distance chaperone for G3P, DA, and AzA and can interact with itself as well as AZI in the process. These SAR signals lead to 4) the accumulation of SA in non-infected tissue via FMO1 and PAD4. Additionally, PIP is induced by ALD1, which amplifies its own synthesis as well as activates SA synthesis. This SA amplification loop leads to 5) NPR1 activation, a key regulator in SAR signaling. Direct binding of SA to NPR1 adapter proteins leads to transcriptional activation of a family of PR genes. PR gene expression is upregulated within 24–48 h in systemic tissues after primary infection. SAR response in non-infected tissue leads to 6) Defense priming, which enables the plant to maintain a state of vigilance to pathogen attack, and react faster and with greater effectiveness. Priming of non-infected, systemic tissue requires MPK3 and MPK6 and leads to defense gene expression as well as heritable effects due to histone methylation and modifications of chromatin. Please note leaves 4 to 6 represent systemic tissues and the depicted processes can occur in any of them, therefore no arrows were used.

Conclusion

Plant hosts have coevolved with their pathogens to form a highly complex immune system. The mechanisms by which plants confer immunity to non-infected tissues have been studied for decades, and yet new factors involved in immune response are being elucidated. One recent development has been the important role that stomata play in the local immune response to plant pathogens. Additionally, the plant hormone SA, which regulates SAR response in non-infected tissues has also been shown to regulate stomatal closure in response to pathogens. Potential SAR long-distance signals have been identified, but it is still unknown how these signals affect different cell types of the systemic tissue as part of immune priming. Future research focusing on molecular changes in different cells and cell types in response to local and distal pathogen infection under various environmental conditions will greatly improve our understanding of the disease triangle and inform rational engineering of crops for enhanced disease resistance without compromising yield.

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