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Systemic acquired resistance: the elusive signal(s)

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Systemic acquired resistance (SAR) is a form of inducible resistance that is triggered in systemic healthy tissues of locally infected plants. The nature of the mobile signal that travels through the phloem from the site of infection to establish systemic immunity has been sought after for decades. Several candidate signaling molecules have emerged in the past two years, including the methylated derivative of a well-known defense hormone (methyl salicylate), the defense hormone jasmonic acid, a yet undefined glycerolipid-derived factor, and a group of peptides that is involved in cell-to-cell basal defense signaling. Systemic SAR signal amplification increasingly appears to parallel salicylic acid-dependent defense responses, and is concomitantly fine-tuned by auxin.

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Introduction

Rooted firmly into their habitat, plants have evolved sophisticated mechanisms to survive the stresses imposed on them by different environments. In many cases, intricate hormonal signaling mechanisms ensure adaptation of the entire plant to a given stress even if only a portion of the plant is exposed. Several kinds of plant–pathogen interactions result in the generation and emission of long-distance signals from the site of infection to healthy uninfected parts of the plant where subsequent resistance is induced: for example beneficial mycorrhizal fungi and root-colonizing rhizobacteria induce pathogen resistance in above-ground plant tissues (reviewed in [1,2]). In addition, infection of plant aerial tissues by biotrophic pathogens results in systemic induction of a long-lasting and broad-spectrum disease resistance referred to as systemic acquired resistance (SAR).

SAR is usually induced by infection of leaves with pathogens that induce hypersensitive cell death (HR; hypersensitive response) owing to resistance (*R*) gene-mediated defense signaling, although an HR is not obligatorily required to generate the long-distance SAR signal [3,4•]. Moreover, basal resistance-inducing pathogen-associated molecular patterns (PAMPs) including the active epitope of flagellin, flg22, induce SAR-like disease resistance [4•]. A recent study showed that SAR further depends on light signaling via the phytochrome receptors PhyA and PhyB [5•]. Whereas SAR signal generation appears to be a general feature of salicylic acid (SA)-dependent defense signaling, the mobile signal itself has been elusive for decades. Several recent major advances towards elucidating the nature of the SAR signal and its systemic amplification are the main focus of this review.

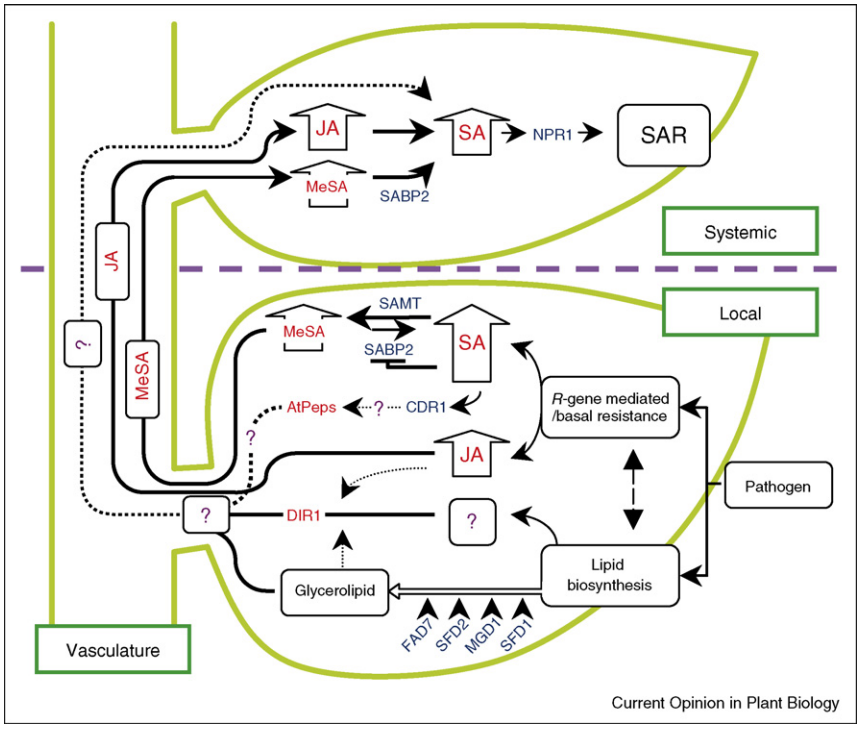
Signal generation and transmission

Methyl salicylate

Accumulation of SA is required for SAR, but only in the signal-perceiving systemic tissue: grafting experiments showed that tobacco leaves infected with tobacco mosaic virus (TMV) could transmit a SAR signal despite the presence of bacterial salicylate hydroxylase (SH) encoded by the *NahG* gene. By contrast, expression of this SA-degrading enzyme in systemic tissue abolished SAR signal perception [6]. Recently, we showed that the SA-derivative methyl salicylate (MeSA) is not degraded by SH *in vitro*, accumulates in *NahG* transgenic tobacco, and acts as a long-distance mobile signal for SAR [7••]. Hydrolysis of MeSA to SA by the MeSA esterase activity of SA-binding protein 2 (SABP2) in the systemic tissue triggers SAR, most likely by initiating the SA positive feedback loop (Figure 1). SA feedback inhibition of SABP2 [8] in the primary inoculated tissue ensures the accumulation of sufficient amounts of the signal, as SAR is abolished when MeSA levels are suppressed in these tissues by expression of an uninhibitable MeSA esterase or by RNAi-mediated silencing of the gene encoding the enzyme that produces MeSA, *SA methyl transferase 1* (*SAMT1*; Figure 1) [7••]. MeSA itself appears to be biologically inactive as it fails to induce defense gene expression or disease resistance in *NahG* transgenic tobacco or in *Arabidopsis* overexpressing a rice methyl transferase for SA and benzoic acid, *OsBSMT1* [9•,10].

Analyses of an 18-member gene family in *Arabidopsis* termed *At methyl esterase 1-18* (*AtMES1-18*) showed that MeSA likely is a conserved SAR signal (AC Vlot, *et al.*, in press). At least five family members displayed MeSA esterase activity *in vitro*, and three of these restored SAR proficiency to SAR-deficient *SABP2*-silenced

Figure 1



Long-distance SAR signaling through phytohormones, lipid metabolites and peptides. Working model of (putative) SAR signaling components, including MeSA, JA, glycerolipid-derived factors and *At*PEPs, and their systemic recognition/amplification. Small molecules are shown in red while proteins/enzymes are in blue. See Section 'Concluding remarks' for details.

tobacco. Furthermore, under expression of MeSA esterases enhanced MeSA accumulation and partially compromised SAR in *Arabidopsis*. In addition to serving as an endogenous SAR signal, MeSA can serve as an airborne signal that is emitted from infected plants and induces defense gene expression in neighboring wild type plants [9•,11]. Taken together, MeSA appears to be a major communication signal for defense both within and between plants.

Lipid signaling

A mutation affecting the lipid-transfer protein DIR1 (DEFECTIVE IN INDUCED RESISTANCE 1) renders *Arabidopsis* incapable of generating/transmitting a functional SAR signal, but does not affect resistance in the inoculated leaf (Figure 1) [12]. The lipid-derived molecule that interacts with DIR1 is unknown, but mutations in several genes encoding enzymes involved in chloroplast galactolipid metabolism (*FAD7*, *SFD1*, *SFD2*, *MGD1*) similarly abolish SAR without affecting basal resistance (Figure 1) [13••,14]. Leaves of infected *sfd1* or *fad7 Arabidopsis* fail to emit a conserved SAR signal that induces defense gene expression or pathogen resistance in *Arabidopsis*, tomato, and/or wheat [13••]. However, petiole exudates from infected *dir1* plants restore systemic defense signaling of comparable exudates from *sfd1* or *fad7* mutants indicating that a glycerolipid-derived factor may interact with DIR1 to trigger SAR.

Another potential lipid-derived SAR signal is the oxylipin-derived defense hormone jasmonic acid (JA), which might be an early signal establishing systemic immunity (Figure 1) [15••]. Early accumulation of JA in phloem exudates and JA-dependent gene expression in the systemic leaves of infected plants correlates with SAR, while SAR is compromised in several JA signaling mutants. Tobacco lipid-transfer protein 1 (LTP1) induces disease resistance, but only when applied to plants together with its ligand JA [16]. Therefore, it was hypothesized that protein–lipid complexes such as LTP1-JA and potentially DIR1-JA are involved in long-distance SAR signaling [15••,16,17]. However, the link between JA and SAR remains unclear since SAR is not altered in all JA signaling mutants [4•,18]. Also, the glycerolipid-derived factor in petiole exudates that apparently induces SAR in conjunction with DIR1 does not co-purify with JA, and JA does not reconstitute an active defense signal in petiole exudates from infected *sfd1* or *fad7* mutants [13••]. Taken together, two lipid-associated signals may work in parallel with each other and MeSA to regulate SAR, but whether one of these signals is a jasmonate derivative has yet to be resolved.

Both galactolipid metabolites and JA could perform dual roles in SAR signal regulation. Accumulation of a set of complex galactolipids, Arabidopsides, carrying JA-precursors 12-oxo-phytodienoic acid (OPDA) and/or dinor-OPDA, is differentially regulated upon wounding or pathogen infection of *Arabidopsis* [19,20,21]. Kourtchenko *et al.* [21] suggested that the level of JA and thereby the nature of its interaction with (Me)SA [15••,22] during pathogen infection and SAR development can be tightly controlled via synthesis and degradation of the HR-associated Arabidopsides E and G. JA in turn induces the expression of genes encoding SA methyl transferases in different plant species thereby enhancing the accumulation of MeSA in *Arabidopsis* and the emission of MeSA from tomato leaves [9•,23,24]. Thus, in addition to its putative independent role in SAR signal transmission, JA induction during pathogen infection [25] strengthens the MeSA component of the SAR signal.

Peptide signaling

The apoplastic aspartic protease CDR1 (CONSTITUTIVE DISEASE RESISTANCE 1) reportedly generates a small peptidic mobile signal that induces systemic defense gene expression in *Arabidopsis* (Figure 1) [26]. The substrate of CDR1 is currently unknown, but it is tempting to speculate that it processes the newly discovered PROPEP proteins into their active peptide forms *At*Pep1-6 [27•,28•]. *PROPEP1-4* are differentially regulated by various defense signals, including MeSA, MeJA and flg22, as well as by their own processed peptides, and the corresponding *At*Peps are hypothesized to support a positive feedback loop amplifying and/or perpetuating PAMP-induced defense signaling (Figure 1) [28•,29]. At least one cell surface, membrane-associated *At*Pep receptor, a receptor-like kinase, has been identified so far [29,30••]. This finding strongly implies a role for the *At*Peps in cell-to-cell defense signaling, but their role in SAR remains to be assessed.

Vasculature-associated signaling

A hypothetical function of nitric oxide (NO) in systemic defense signaling [31] was recently reinforced in a study linking the level of protein *S*-nitrosylation, that is the formation of *S*-nitrosothiols (SNOs), to SAR [32•]. SNO levels were induced in both infected and systemic tissues of SAR-induced *Arabidopsis*, and suppression of SNO accumulation by over expression of *S*-nitrosoglutathione reductase (GSNOR) compromised SAR. Since GSNOR is localized to phloem companion cells and xylem parenchyma, and GSNOR over expressing plants accumulated elevated levels of it in their vascular system, it was hypothesized that GSNOR plays a role in SAR signal transport through the vasculature [32•,33]. In support of this notion, NO is induced in phloem of *Vicia faba* after treatment with H_2O_2 or SA, while phloem exudates of H_2O_2-treated *Cucurbita maxima* contains elevated levels of nitrated proteins [34]. By contrast, Feechan *et al.* [35] noted an inverse correlation between SNO levels and both basal and *R* gene-mediated resistance. Though contradictory, these findings suggest that SNOs might play a role in SAR signaling, but their mechanism of action is unclear.

Other signals that are less well characterized in the context of SAR signaling are generated by MAP kinase signaling cascades. For instance, MAP Kinase Kinase 7 (MKK7), a negative regulator of polar auxin transport, is involved in basal resistance and SAR [36••]. Expression of *MKK7* localizes exclusively to the vasculature of infected *Arabidopsis* leaves, consistent with a putative role in SAR signal transmission. Moreover, conditional over expression of *MKK7* induces defenses in both the over expressing and systemic, non-*MKK7*-expressing tissues [36••]. The demonstration that *MKK7* expression is upregulated by HR-inducing bacteria further supports a role in SAR signal generation/transmission.

By contrast, the MAP Kinase MPK4 was hypothesized to be a negative regulator of SAR [37]. Recent genetic analyses suggest that MPK4 regulates both SA signaling and the JA/ethylene defense pathways via EDS1 and PAD4 [38]. Thus, a specific role for MPK4 in generating/transmitting the systemic SAR signal seems unlikely. However, the MAP Kinase Kinase Kinase MEKK1, which is involved in PAMP-mediated defense signaling [39,40,41], activates MPK4 in a mechanism that is independent of MEKK1 kinase activity [39,40]. Interestingly, the activities of both MPK3 and MPK6, well-established SA-mediated defense regulators, are enhanced in the *mekk1* mutant [39]. Moreover, expression of *MEKK1*, with the exception of guard cells, localizes predominantly to the vascular tissue of *Arabidopsis* leaves, while (HR) cell death and hydrogen peroxide accumulation occur in the vasculature and/or guard cells of the *mekk1* mutant [39]. Together, the data argue in favor of an antagonistic role of MEKK1 and MPK4 signaling on MPK3 and MPK6, possibly affecting SAR signal transmission through the vasculature.

Signal perception and amplification

SAR and SA-mediated defense signaling partially overlap [42] since the SA positive feedback loop is essential for amplifying the SAR signal in systemic tissues. *NON EXPRESSOR OF PR-1* (*NPR1*) is one of the main regulators of SA and SAR signaling (Figure 1), and its functions have been extensively reviewed elsewhere (e.g. [17,43]). Accumulating evidence suggests that SA and auxin perform mutually antagonistic roles in disease resistance [44,45••], and repression of auxin-related genes was observed in the systemic tissue of SAR-induced *Arabidopsis* [45••]. Recently, members of the GH3 family of acyl-adenylate/thioester-forming enzymes involved in the amino acid conjugation of, for example the auxin indole-3-acetic acid (IAA), were implicated in the

regulation of basal and *R* gene-mediated resistance as well as SAR [46–48,49,50•]. GH3.5 can conjugate both SA and IAA [51], and both signaling pathways were upregulated in plants over expressing GH3.5 after pathogen infection [50•]. In spite of heightened SA accumulation and *PR* gene expression, *R* gene-mediated resistance in these plants was suppressed, presumably owing to the enhanced susceptibility conferred by elevated IAA levels. In *gh3.5* mutants, SAR was partially compromised as indicated by suppressed *PR-1* expression in systemic tissues [50•]. It should be noted that in an independent study, over expression of GH3.5 led to elevated SA levels and *PR-1* transcripts and suppression of IAA levels [49]. Another member of the *GH3* family, *GH3.12*, is required for SA-mediated disease resistance; mutations in this gene (*pbs3*, *gdg1*, *win3*) appear to suppress SA and/or SA-glucoside accumulation and confer enhanced susceptibility to avirulent and virulent *Pseudomonas*, and/or suppress SAR, although not all of the results are consistent among these studies [46–48]. Identifying the substrates of defense-related GH3 acyl adenylases, including GH3.5 and GH3.12, might shed light on the mechanism(s) through which auxin and SA signaling perturb each other to establish either susceptibility or resistance.

Concluding remarks

Figure 1 summarizes SAR signaling in a model encompassing the different components that together may constitute the mobile SAR signal(s). MeSA and the different lipid-derived components each appear to be conserved across plant genera ([7••,13••,15••,16], AC Vlot, *et al.*, in press); genetic manipulations which affect singular components abolish SAR in the pathosystems studied to date. A major future challenge will be to determine how the different factors interact to facilitate their integration into a signaling network. An additional challenge involves translating this knowledge into practical applications. A recent field study confirmed that SAR increases the fitness of plants exposed to pathogens, which translates into enhanced crop yield [52]. However, unlike the fitness cost of constitutive resistance that associated with inducible resistance generally appears to outweigh the cost of pathogen infection, although this might depend on additional environmental factors [53,54•]. In the era of metabolomics, large-scale surveys might reveal additional candidate compounds involved in SAR induction (e.g. [55]); perhaps both established and new signals can be used to enhance the natural defenses of crop plants while retaining optimal yield.

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pathogen infection, but did not mount a significant defense response to pathogen infection or when treated with SA, the data confirm earlier findings that MeSA is not biologically active in defense [10]. Expression of *PR-1* was induced in both wild type plants and in SA-deficient *sid2-2* mutants when they were maintained in a container with *OsBSMT1*-over expressing plants that were treated with SA. Together, the data strengthen earlier findings that MeSA can act as an airborne, plant-to-plant defense signal that is converted to SA in the signal-perceiving plant [11].

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In addition to a mutation in the previously published *SFD1* gene (*SUPPRESSOR OF FATTY ACID DESATURASE 1*) [14], mutations in three other genes involved in chloroplast galactolipid metabolism, *FAD7* (*FATTY ACID DESATURASE 7*), *MGD1* (*MONOGALACTOSYLDIACYLGLYCEROL SYNTHASE 1*), and *SFD2*, abolished SAR without affecting basal resistance or the accumulation of SA in leaves infected with avirulent *P. syringae*. Petiole exudates from leaves of *sfd1* or *fad7 Arabidopsis* infected with avirulent *P. syringae* failed to induce defense gene expression or pathogen resistance in *Arabidopsis*, tomato, and/or wheat, unlike those from comparable wild type plants. As defense signaling was restored by combining these petiole exudates with similar exudates from *dir1* mutant plants, which lack a functional form of the lipid-transfer protein DIR1 and also do not generate or transmit the SAR signal [12], it was concluded that a glycerolipid-derived factor and DIR1 may interact or act in parallel to trigger SAR. The glycerolipid-derived factor did not co-elute with JA from a gel filtration column, and JA did not restore the defense signaling potential of petiole exudates from infected *sfd1* or *fad7* mutant plants, indicating that the glycerolipid-derived SAR signal is not JA.

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the *At*Pep1 receptor, PEPR1, is a membrane-associated leucine-rich repeat receptor-like kinase. Membrane fractions isolated from T-DNA insertional mutants in the *PEPR1* gene were incapable of binding ^{125}I-*At*Pep1, while over expression of the receptor caused hypersusceptibility of tobacco suspension cells to *At*Pep1. PEPR1 interacted with four of six *At*Pep1 homologues that were tested in competitive binding assays with ^{125}I-*At*Pep1. The association of PEPR1 with the cell surface strongly argues for a role of the *At*Peps in cell-to-cell defense signaling.

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The level of *S*-nitrosothiols (SNOs) was manipulated by over and under expression of *S*-nitrosoglutathione reductase (GSNOR). A 45% reduction of GSNOR activity resulted in approximately a twofold increase of SNOs, while over expression of GSNOR activity by 19-fold reduced SNO levels by 20%. SAR correlated with elevated levels of SNOs in both the infected and systemic tissues of wild type plants, and this induction was not observed in the *GSNOR* over expressor. Depending on the pathogen used, basal resistance was enhanced in the *GSNOR*-silenced line. The slight reduction of the SNO level in the *GSNOR* over expressor did not significantly affect basal or *R* gene-mediated resistance, but severely compromised SAR.

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Arabidopsis over expressing *MKK7* displayed constitutive accumulation of SA and SA-related defense gene transcripts, as well as enhanced resistance to pathogens. These phenotypes depended on the kinase activity of MKK7 as they did not occur in plants over expressing a kinase-inactive mutant. Analysis of *MKK7::GUS* reporter lines showed that *MKK7* is expressed exclusively in the veins of leaves that have been infected with avirulent *P. syringae*. Interestingly, conditional expression of *MKK7* from a dexamethasone-inducible transgene triggered defense gene expression and pathogen resistance in systemic non-*MKK7* expressing tissues. Together, the data strongly argue that the MKK7 kinase is involved in SAR signal generation and/or transmission.

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Micro array analyses showed that the SA analog BTH induces several auxin-conjugating enzymes and represses a large group of auxin-response genes, most of which are also repressed in systemic tissues of SAR-induced plants. These transcriptional changes were not paralleled by changes in auxin levels. The morphological phenotypes of an auxin over producing mutant were compromised by introducing mutations which cause constitutive SA signaling, although auxin levels remained high. Reporter gene studies confirmed that SA antagonizes auxin signaling (rather than its metabolism) via SA-dependent stabilization of auxin repressor proteins. By contrast, application of an auxin enhanced susceptibility of *Arabidopsis* to pathogens whereas an auxin-insensitive mutant displayed enhanced resistance to *P. syringae*. Since insensitivity to auxin partially rescued the hypersusceptible phenotype of *Arabidopsis* expressing the SA-degrading enzyme encoded by *NahG*, the authors concluded that SA enhances resistance by inhibiting auxin signaling.

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GH3.5 can conjugate auxin and SA [51], and over expression of *GH3.5* resulted in elevated auxin and SA levels after pathogen infection of *Arabidopsis*. In spite of enhanced SA levels, *R* gene-mediated resistance was compromised in the *GH3.5* over expressor. A comparison of this disease resistance phenotype with that of *Arabidopsis* over expressing the closest homolog of *GH3.5*, *GH3.6*, which can only conjugate auxin and not SA, argued that elevated auxin levels likely antagonized SA signaling, which resulted in increased susceptibility. Basal and *R* gene-mediated resistance were not affected in the *gh3.5* knock out mutant, but SAR was slightly compromised, which was accompanied by reduced systemic induction of *PR-1*. *GH3.5* expression was induced by pathogens and SA, and transcript profiling of the *GH3.5* over expressor revealed that GH3.5 might enhance IAA biosynthesis and activate auxin signaling. Expression of SA-dependent and other defense genes was also higher in the over expressor than in wild type plants, particularly after pathogen infection.

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This paper directly compares the effects of low and high concentrations of the defense-inducing compounds β-aminobutyric acid (BABA) and the SA analog BTH on pathogen resistance and plant growth/yield. Low concentrations of the compounds induced SAR-like priming of defense pathways that were activated faster and/or stronger than in non-treated plants upon pathogen infection. Higher concentrations of the compounds constitutively activated defense. The data show that defense through priming is equally effective as constitutively activated defense. However, contrary to constitutive activation of defense, priming did not cause significant plant growth retardation or loss of seed production. As plant growth and seed production were not affected in *npr1* mutant plants after pathogen infection or treatment with high concentrations of BABA or BTH, it was concluded that the 'costs' of infection/constitutive defense were owing to *NPR1*-dependent defense responses.

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