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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**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 SAR proficiency to SAR-deficient *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 methyltransferase 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...
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 copurify 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 AαPep1-6 [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 AαPeps 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 AαPep receptor, a receptor-like kinase, has been identified so far [29,30***]. This finding strongly implies a role for the AαPeps in cell-to-cell defense signaling, but their role in SAR remains to be assessed.

**Vascularization-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 plasmolysis 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 plasmolysis of Vicia faba after treatment with H2O2 or SA, while plasmolysis exudates of H2O2-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 M KK7 localizes exclusively to the vasculature of infected Arabidopsis leaves, consistent with a putative role in SAR signal transmission. Moreover, conditional over expression of M KK7 induces defenses in both the over expressing and systemic, non-MKK7-expressing tissues [36**]. The demonstration that M KK7 expression is upregulated by HR-inducing bacteria further supports a role in SAR signal generation/transport.

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-I 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-I 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, gdl1, 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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References and recommended reading

Papers of particular interest, published within the period of the review, have been highlighted as:

- of special interest
- of outstanding interest


This paper shows that virulent and non-host Pseudomonas–Arabidopsis interactions trigger a SAR response accompanied by systemic induction of SA and defense gene expression. As these responses were compromised in known SAR mutants, the authors concluded that the non HR-inducing bacteria induced true SAR. Localized application of PAMPs, including flg22, triggered very limited local and systemic induction of SA and defense gene expression, but non-recessive induced a state of significantly heightened resistance to virulent P. syringae in the systemic tissue.


Data in this paper show that the development of both a visible HR and SAR correlates with the length of time that plants receive light immediately following infection of Arabidopsis with avirulent P. syringae. End-of-day inoculations resulted in delayed PR-1 induction and compromised SA accumulation in inoculated leaves as compared to start-of-day inoculations. The photoreceptor mutants cry1cry2, phot1phot2, and phyAphyB all displayed normal local defence responses, although SA accumulation and defense against virulent P. syringae might be partially compromised in the phyAphyB mutant. Contrary to wt-like SAR in cry1cry2 and phot1phot2 plants, SAR was completely abolished in the phyAphyB mutant.


Grafting experiments showed that the tobacco MeSA esterase SABP2 is required in the signal-perceiving/processing tissue, but not in the signal-generating tissue, to trigger SAR. The SAR-deficient phenotype of SABP2-silenced graft scions was complemented by expression of SABP2, but only if it was capable of converting MeSA to SA. Feedback inhibition of the MeSA esterase activity of SABP2 [8] in the signal-generating tissue is of biological significance as expression of a form of SABP2 with uninhitable MeSA esterase activity in the graft rootstock abolished SAR. Silencing of the gene encoding a SA methyl transferase, SAMT7, similarly abolished SAR signal generation in graft rootstocks. Increases in MeSA levels in TMV-inoculated and systemic tissues, as well as in petiole (phloem) exudates of infected leaves, paralleled the development of SAR.


A nice carboxyl methyl transferase for SA and benzoic acid, OsBSMT1, was expressed in Arabidopsis. Since the transgenic plants accumulated elevated levels of MeSA and methyl benzoate, particularly in response to
pathogen infection, but did not mount a significant defense response to pathogen infection, or when treated with SA. Together, these 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, these 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].


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 (MONOGLYCOSYLDIACYLGlycerol synthase 7), 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 sfd7 Arabidopsis infected with avirulent P. syringae failed to induce defense gene expression or pathogen resistance in Arabidopsis, tomato, and/or wheat, like those from comparable wild type plants. As defense signaling was restored by combining these petiole exudates with sfd1 or sfd7 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-purify with JA from a gel filtration column, and JA did not restore the defense signaling potential of petiole exudates from infected sfd1 or sfd7 mutant plants, indicating that the glycerolipid-derived SAR signal is not JA.


A previously identified early PAMP-induced gene was transiently induced in systemic Arabidopsis tissues, peaking at around four hours after a localized infection of the plant with avirulent P. syringae. This observation was followed up with micro array analyses of systemic tissue of SAR-induced and control plants at four hours after infection. Genes of the phenylpropanoid pathway were strongly induced, and the authors suggested that phenolic compound-related defenses were primed. However, most of the systemically upregulated genes had roles in JA biosynthesis or had previously been characterized as JA responsive. JA was found in petiole exudates of infected leaves, and the JA biosynthetic mutant opr3 and the JA-insensitive mutant jin7 were SAR deficient. JA may therefore function as an early mobile signal triggering SAR. The authors hypothesized that JA and SA might both affect SAR in a temporally or spatially separated manner.


This paper describes the characterization of a small 23 amino acid peptide (AtPEP1) that induces accumulation of H2O2. JA-dependent defense gene expression, and expression of the gene encoding its own precursor, PROPEP1. PROPEP1 was also induced by MeJA, indicating the existence of a positive feedback loop. Together with five homologues, PROPEP1 makes up a small gene family, with PROPEP2 and 3 being strongly activated by pathogens which are capable of inducing SAR. Over expression of PROPEP1 in Arabidopsis induced resistance to the soil-borne fungal pathogen Pythium irregulare.


This paper uses semi-quantitative reverse transcriptase PCR to characterize the expression of different PROPEP family members in response to MeSA, MeJa, and the PROPEP-encoded, processed AtPEP peptides. PROPEP1, 2, and to a lesser extent 4, were induced by MeJa, whereas PROPEP2 and 3 were induced by MeSA. Expression of PROPEP1 was induced by AtPEP1, whereas expression of PROPEP2 and 3 was induced by multiple AtPEPs. The positive feedback loop observed in [27] was also seen here as AtPEP1 and 2 strongly induced expression of the JA-responsive PDF1.2 gene, and AtPEP1, 2, 3, 5, and 6 strongly activated expression of the SA-responsive PR-1 gene. Since PAMPs, such as flg22, induce PROPEP2 and 3, the authors proposed that the PROPEP gene family is involved in defense signaling by establishing a positive feedback loop.


In this paper, the AtPEP1 receptor was purified from Arabidopsis suspension cells using 125I-labeled AtPEP1. Similar to known PAMP receptors,
the AtPEP1 receptor, PEPR1, is a membrane-associated leucine-rich repeat receptor-like kinase. Membrane fractions isolated from T-DNA insertion mutants in the PEPR1 gene were incapable of binding 125I-AtPEP1, while over expression of the receptor caused hypersusceptibility of tobacco suspension cells to AtPEP1. PEPR1 interacted with four of six Arabidopsis homologues that were tested in competitive binding assays with 125I-AtPEP1. The association of PEPR1 with the cell surface strongly argues for a role of the AtPEPs in cell-to-cell defense signaling.


The level of S-nitrosi-thiols (SNOS) was manipulated by over and under expression of S-nitrosogluthathione 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 SNOS 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.


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.


Arabidopsis over expressor background. A comparison of this GH3.5 over expressor to GH3.5 silence line. The expression of the SA-degrading enzyme encoded by NahG, the authors concluded that SA enhances resistance by inhibiting auxin signaling.


Micro array analyses showed that the SA analog BTH induces several auxin-coordinated enzymatic processes, 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 auxin expression, the authors concluded that SA enhances resistance by inhibiting auxin signaling.


50. 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.


This paper directly compares the effects of low and high concentrations of the defense-inducing compounds \( \beta \)-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.