# Interconnection between Methyl Salicylate and Lipid-Based Long-Distance Signaling during the Development of Systemic Acquired Resistance in Arabidopsis and Tobacco[1][W]

**Po-Pu Liu, Caroline C. von Dahl, Sang-Wook Park[2], and Daniel F. Klessig***

Boyce Thompson Institute for Plant Research, Ithaca, New York 14853



Systemic acquired resistance (SAR) is a salicylic acid (SA)-dependent heightened state of resistance against a broad spectrum of pathogens activated in the uninoculated systemic tissue of a pathogen-infected plant. For systemic protection to be initiated, a mobile signal that is produced at the site of primary infection needs to travel through the plant. Although the mobile signal (s) for SAR has been the subject of considerable research over several decades, its identity remains controversial. Our analyses of Arabidopsis (*Arabidopsis thaliana*) and tobacco (*Nicotiana tabacum*) *defective in induced resistance1* (*dir1*) mutants, which are unable to develop SAR, reveal a connection between two candidate mobile signals: methyl salicylate (MeSA) and the so-far-unidentified lipid-derived signal or signal complex missing in the *dir1* mutant.

The demonstration that SA accumulates in the phloem and is required to activate SAR led to the proposal of SA as the mobile signal (Yalpani et al., 1991). However, grafting experiments with tobacco plants expressing the bacterial *NahG* gene, which encodes the SA-degrading enzyme SA hydroxylase, suggested otherwise. Tobacco mosaic virus (TMV)-infected NahG rootstocks were still capable of generating the signal for induction of SAR in wild-type scions, despite their inability to accumulate SA. Strikingly, *NahG*-expressing scions failed to develop SAR, arguing that SA was essential for SAR development in the healthy systemic tissue (Vernooij et al., 1994). The requirement of SA in the systemic tissue for SAR development is now well established (for review, see Vlot et al., 2008a). Subsequent studies involving quantification of MeSA and SA levels, along with characterization of grafted plants in which the genes responsible for MeSA biosynthesis (*SA methyltransferase1* [*SAMT1*]) or MeSA cleavage to SA (*SA-binding protein2* [*SABP2*]) were silenced in the rootstock or scion, suggested that MeSA is a mobile SAR signal in tobacco. Analysis of these chimeric tobacco plants indicated that NtSAMT1 activity, and thus MeSA biosynthesis, is required in the primary infected leaves where the SAR signal is produced. In contrast, MeSA esterase (MSE) activity is needed in the uninoculated systemic leaves, where the SAR signal is perceived and processed (Park et al., 2007). MeSA does not induce defense responses (Seskar et al., 1998); instead, it must be converted to SA by a MSE for biological activity. Further research revealed that SABP2's MSE activity must be inhibited in the primary infected tissue (by SA binding in its active site pocket) to facilitate the accumulation of sufficient levels of MeSA to signal SAR (Park et al., 2007, 2009). Subsequent characterization of MSEs in Arabidopsis and potato (*Solanum tuberosum*) confirmed the relevance of MSE activity for SAR signaling in these two species (Vlot et al., 2008b; Manosalva et al., 2010). The demonstration that 2,2,2,2′-tetrafluoracetophenone, a synthetic SA analog that inhibits MSE activity, blocks SAR development in tobacco, Arabidopsis, and potato further confirmed the importance of this enzyme and MeSA for SAR development in these plant species (Park et al., 2009).

Other candidate mobile signals can be broadly categorized as lipid based/related. The first link between lipid synthesis and SAR came from analyses of the Arabidopsis *suppressor of SA insensitivity2* (*ssi2*) mutant. These plants constitutively exhibit SAR and fail to convert stearic acid (18:0) to oleic acid (18:1) because of a mutation in a stearoyl-acyl carrier protein desaturase (Kachroo et al., 2001). Interestingly, the *ssi2* suppressor mutant *suppressor of fatty acid desaturase1* (*sfd1*; also known as *gly1* [Kachroo et al., 2004]), which is compromised for SAR but not for local resistance against virulent and avirulent pathogens, contains a mutation in an enzyme involved in the metabolism of glycerol-3-P, the backbone of glycerolipids (Browse and Somerville, 1991; Nandi et al., 2003, 2004). Further evidence of a lipid-derived SAR signal came from characterization of the *dir1-1* Arabidopsis mutant. These plants contain a mutation in a putative apoplastic protein with ho-

[1] This work was supported by the National Science Foundation (grant nos. IOS–0525360 and IOS–0820405 to D.F.K.) and a German Research Foundation fellowship (grant no. DA1239/1–1 to C.C.v.D.).

[2] Present address: Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

* Corresponding author; e-mail dfk8@cornell.edu.

[W] The online version of this article contains Web-only data.

www.plantphysiol.org/cgi/doi/10.1104/pp.110.171694

mology to family 2 lipid transfer proteins and they are compromised for SAR but not for local resistance. Demonstrating the importance of DIR1 for SAR signaling, phloem/petiole exudates (PEXs) from the infected leaves of wild-type plants, but not *dir1-1* plants, induced *PR-1* transcript accumulation and enhanced resistance against virulent *Pseudomonas syringae* following infiltration into *dir1-1* leaves (Maldonado et al., 2002). Subsequent studies revealed that combined PEXs from pathogen-infected *dir1-1* mutants and *sfd1* mutants induced SAR in infiltrated wild-type plants, while neither mutant PEX alone was sufficient to elicit SAR (Chaturvedi et al., 2008). These results suggest that at least two components work in conjunction: (1) most likely a protein (DIR1) that is functional in PEX from infected *sfd1* mutants and (2) a lipid derivative that is present in PEX from infected *dir1-1* plants. The identity of these proposed lipid-based signaling components remains elusive. However, other studies have identified specific lipids as candidate mobile signals, namely jasmonic acid and azelaic acid (Truman et al., 2007; Jung et al., 2009). Whereas azelaic acid is suggested to act downstream of SFD1 and independent of DIR1, jasmonic acid's role in SAR is highly controversial (Shah, 2009).

To investigate the relationship between lipid- and MeSA-based, long-distance SAR signaling, we monitored MeSA levels, *AtBSMT1* expression, and SAR development in the *dir1-1* mutant (for details, see Supplemental Materials and Methods S1). Surprisingly, *dir1-1* not only failed to develop SAR (Maldonado et al., 2002; Chaturvedi et al., 2008), but also exhibited increased levels of MeSA and *AtBSMT1* transcripts in both *P. syringae* pv *maculicola* (*Psm*)-infected leaves and uninoculated systemic tissue, as compared with wild type (Fig. 1, A and B). Increases in *AtBSMT1* expression were readily detected in wild-type plants by 24 h postinfection (hpi) with avirulent *Psm AvrRpt2 cor*$^-$. By contrast, *dir1-1* plants displayed earlier and stronger *AtBSMT1* expression; increases were readily detected by 12 hpi,

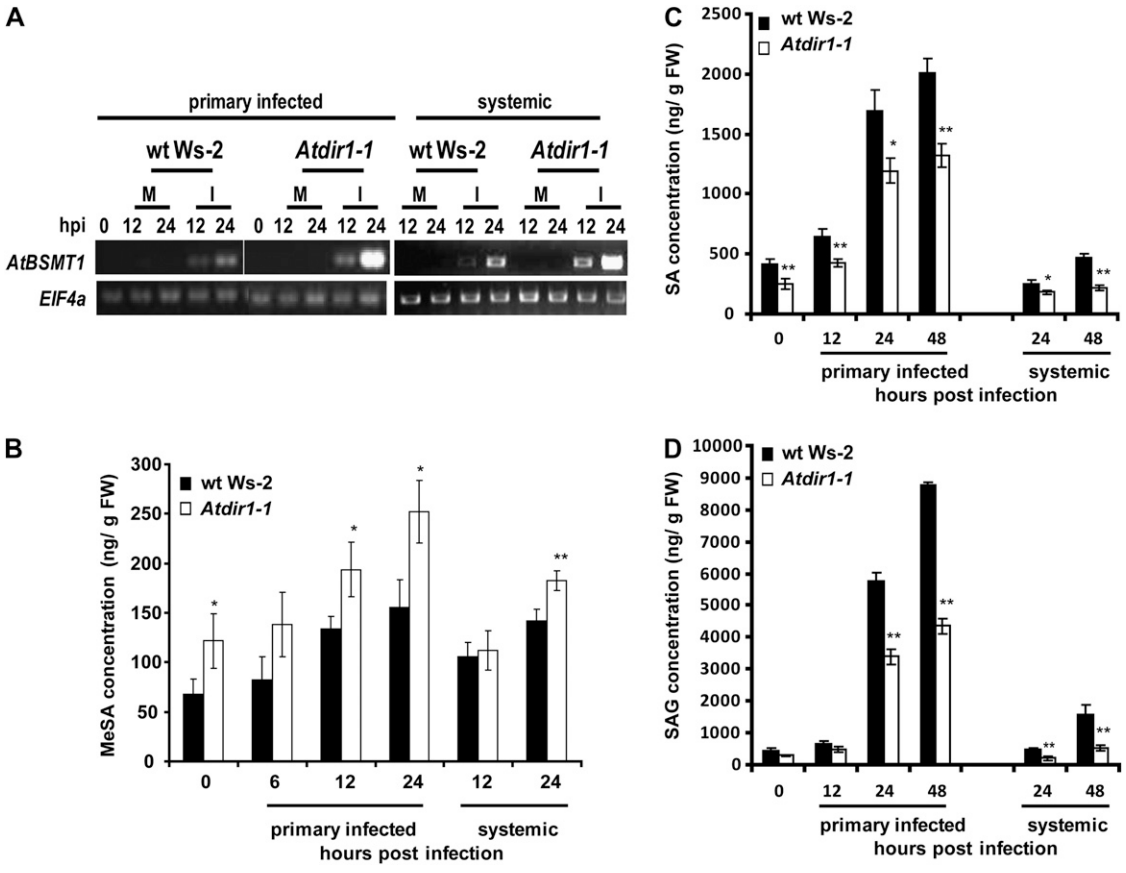
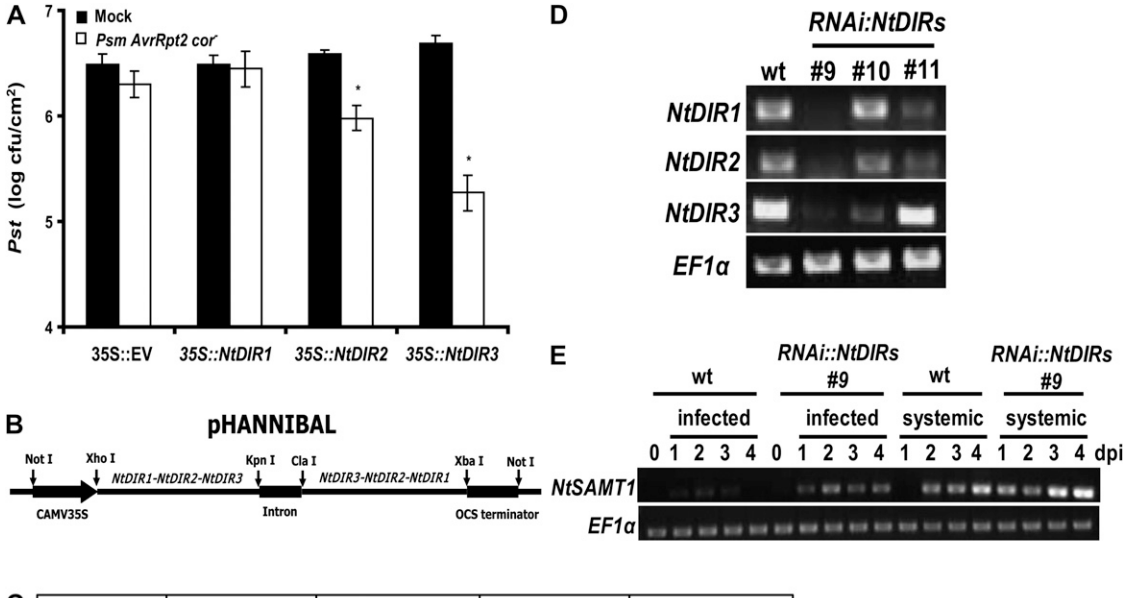



**Figure 1.** Characterization of wild-type Ws-2 and *Atdir1-1* plants. A, *AtBSMT1* gene expression in primary infected and systemic leaves was analyzed at the indicated times in hours postmock (M) or *Psm AvrRpt2 cor*$^-$ (I) infection of 3- to 4-week-old wild-type Ws-2 or *Atdir1-1* plants. *Eukaryotic Translation Initiation Factor4a* (*EIF4a*) was used as an internal control in the reverse transcriptase-PCR assays. *AtBSMT1* and *EIF4a* were amplified using 25 and 20 cycles, respectively. Two independent experiments were analyzed with similar results. B, MeSA. C, SA. D, SAG contents were quantified in primary infected and systemic leaves of *Psm AvrRpt2 cor*$^-$-infected 3- to 4-week-old plants at the indicated times. Asterisks in B, C, and D indicate statistically significant differences (* = $P < 0.05$, ** = $P < 0.01$, Student's *t* test) between levels in *Atdir1-1* versus wild-type Ws-2 for each time point. Mean ± SD values are presented. Two independent experiments were analyzed with similar results.




C

| | EV | | RNAi::NtDIRs # 9 | | RNAi::NtDIRs # 10 | | RNAi::NtDIRs #11 | |
|---|---|---|---|---|---|---|---|---|
| 1° treatment | Mock | TMV | Mock | TMV | Mock | TMV | Mock | TMV |
| 2° TMV lesion size (mm ± SD) | 3.11 ± 0.16 | 1.64 ± 0.34 | 3.12 ± 0.23 | 3.22 ± 0.28 | 2.86 ± 0.20 | 2.78 ± 0.23 | 3.10 ± 0.15 | 1.71 ± 0.18 |
| Reduction (%) 2° lesion size to Mock | N/A | 47 | N/A | -3 | N/A | 3 | N/A | 45 |
| SAR | N/A | + | N/A | - | N/A | - | N/A | + |

**Figure 2.** Characterization of the tobacco homlogs of AtDIR1. A, Restoration of SAR proficiency in *Atdir1-1* by CaMV 35S-driven expression of *NtDIR1*, *NtDIR2*, and *NtDIR3*. T2 transgenic plants were used for SAR analysis. The CaMV 35S-driven EV served as the control. Asterisks indicate statistically significant differences (* = $P < 0.01$, Student's *t* test) in the growth of virulent *Pst* in plants induced for SAR by prior infection with *Psm AvrRpt2 cor*⁻ as compared to plants that received an initial mock inoculation and therefore were not induced for SAR. Mean ± SD values are presented. B, The pHANNIBAL construct for silencing of *NtDIR1*, *NtDIR2*, and *NtDIR3* contains approximately 300 nucleotides of each of the three NtDIRs arranged sequentially. C, Analysis of SAR in three transgenic lines, *RNAi::NtDIRs #9*, *RNAi::NtDIRs #10*, and *RNAi::NtDIRs #11*, which contain a single insertion of the pHANNIBAL RNAi construct shown in B. SAR was determined as percent reduction in the size of TMV lesions formed after a secondary infection on plants induced for SAR by prior TMV infection, as compared to lesions formed on plants that had received a mock inoculation and therefore were not induced for SAR. Plants transformed with the pHANNIBAL *RNAi::EV* served as a control. D, The silencing efficiency of *NtDIRs* in *RNAi::NtDIRs #9*, *RNAi::NtDIRs #10*, and *RNAi::NtDIRs #11*. The same plants were used in C. *Elongation factor 1α* (*EF1α*) was used as an internal control in reverse transcriptase-PCR assays. *NtDIR1*, *NtDIR2*, and *NtDIR3* were amplified using 25 cycles, while 20 cycles was used for *EF1α*. E, *NtSAMT1* gene expression was analyzed in the primary infected and systemic leaves of 6-week-old wild-type and *RNAi::NtDIRs #9* plants at the indicated times. *NtSAMT1* and *EF1α* were amplified using 36 and 25 cycles, respectively. Two independent experiments were analyzed with similar results in A, C, D, and E.

and transcript levels continued to rise by 24 hpi (Fig. 1A). This heightened *AtBSMT1* expression correlated with elevated MeSA levels in both the inoculated and systemic leaves of *dir1-1* as compared to wild-type plants at 24 hpi (Fig. 1B). Based on these results, the *DIR1*-mediated mobile signal may function, at least in part, by regulating MeSA biosynthesis. This possibility suggests a potential mechanism for the loss of SAR in *dir1-1* plants. Given the elevated expression of *AtBSMT1* in their systemic leaves, the equilibrium between MeSA and SA would be strongly shifted toward MeSA; this in turn could preclude the accumulation of sufficient levels of SA and its glucoside (SAG) to activate SAR. Consistent with this hypothesis, SA and SAG levels were significantly lower in the systemic leaves of *Psm*-inoculated *dir1-1* plants as compared with wild-type plants at both 24 and 48 hpi (Fig. 1, C and D). SA and SAG levels also were significantly reduced in the *Psm*-infected leaves of *dir1-1*. It is interesting to note that Arabidopsis overexpressing *AtBSMT1* accumulate elevated levels of MeSA, display reduced levels of SA and SAG in their infected and systemic tissue as compared to wild type, and fail to develop SAR (Liu et al., 2010). The similarities between this phenotype and that of *dir1-1* argue that in both plants, the loss of SAR is due to elevated *AtBSMT1* expression in

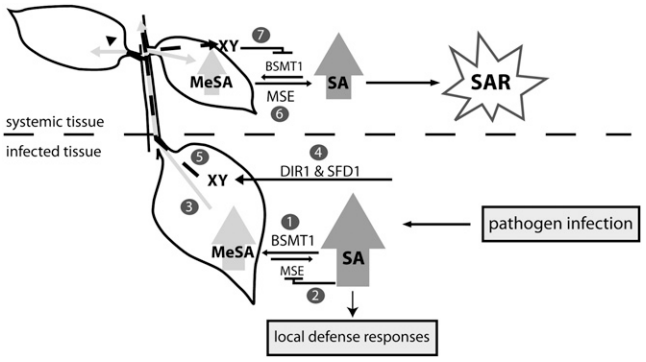



**Figure 3.** Working model of the interrelationship between MeSA and lipid-based, long-distance signaling for SAR. After pathogen attack, SA levels rise in the primary infected tissue. SA is partially converted to MeSA by AtBSMT1 (1). MSE can demethylate MeSA to reform SA; however, the rising levels of SA inhibit MSE by binding in its active site pocket (2). The accumulating MeSA is translocated to the uninoculated systemic tissue (3). In the primary infected tissue, AtDIR1 and an AtSFD1-dependent lipid form a complex, denoted XY (4), which is translocated to the systemic tissue (5). In the systemic tissue, MSE activity converts biologically inactive MeSA to active SA (6). To facilitate SA accumulation, and thereby SAR development, AtBSMT1 expression is directly or indirectly suppressed by the DIR1-lipid derivative complex, XY (7).

systemic tissue, despite heightened production of the candidate mobile SAR signal MeSA in the infected tissue.

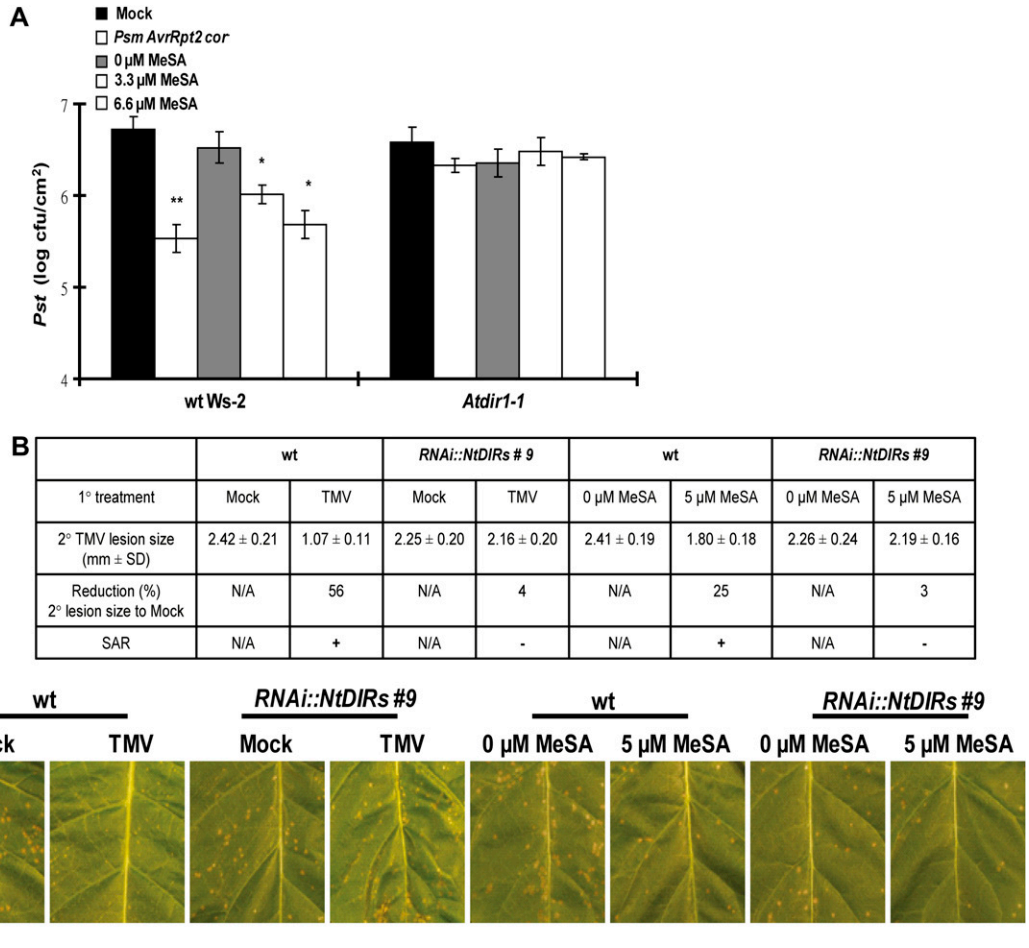
To assess whether tobacco (Solanaceae), like Arabidopsis (Brassicaceae), utilizes a lipid-related SAR signaling system, we initially characterized DIR1 in tobacco. To this end three tobacco AtDIR1 homologs were identified and designated NtDIR1, NtDIR2, and NtDIR3. The shared sequence identity of NtDIR1, NtDIR2, and NtDIR3 to AtDIR1 was 43%, 40%, and 46%, with an overall similarity of 58%, 60%, and 66%, respectively (Supplemental Figs. S1, A and B, and S2). For complementation analyses, each of the tobacco genes was expressed under the control of the constitutive cauliflower mosaic virus (CaMV) 35S promoter in *dir1-1* Arabidopsis (Supplemental Fig. S1C). *dir1-1* transformed with the empty vector (EV) construct (*35S::EV*) failed to develop SAR in response to either mock inoculation or a primary infection with *Psm AvrRpt2 cor*$^-$. Regardless of the primary treatment, comparable levels of bacterial growth were observed in the *35S::EV* plants following a secondary inoculation with virulent *P. syringae* pv *tomato* (*Pst*; Fig. 2A). By contrast, expression of *NtDIR2* or *NtDIR3*, but not *NtDIR1*, restored SAR in *dir1-1* (Fig. 2A), as evidenced by the reduced growth of *Pst* following a primary inoculation with *Psm AvrRpt2 cor*$^-$. This result argues that NtDIR2 and NtDIR3 are orthologous to AtDIR1.

After verification of NtDIR's function in Arabidopsis, we assessed SAR development in *NtDIR*-silenced tobacco. The tobacco *DIR* genes were silenced using a construct containing sequences from all three genes (Fig. 2B). Three of 16 transformed T1 plants, *RNAi*::*NtDIRs #9*, *RNAi*::*NtDIRs #10*, and *RNAi*::*NtDIRs #11*, carried a single insertion of the transgene and displayed considerably reduced transcript levels for at least one *NtDIR* gene (Fig. 2D; data not shown). In the TMV-tobacco pathosystem, development of SAR is manifested by the development of much smaller hypersensitive response lesions after a secondary infection versus those produced after a primary infection. This reduction in lesion size is due to the heightened resistance developed by tobacco plants following a primary infection, which in turn allows them to restrict viral replication and spread more efficiently the second time they encounter the virus. Analysis of secondary lesion sizes in *NtDIR*-silenced tobacco and EV control plants that had received a primary inoculation with either TMV or buffer revealed that SAR was compromised in *RNAi*::*NtDIRs #9* and *RNAi*::*NtDIRs #10*, but not in *RNAi*::*NtDIRs #11* (Fig. 2C). Comparison of the gene-silencing efficiency of *NtDIR1*, *NtDIR2*, and *NtDIR3* (Fig. 2D) with the SAR phenotype revealed a correlation between silencing of *NtDIR3* and loss of SAR. In line *RNAi*::*NtDIRs #9*, SAR failed to develop and all three *NtDIR*s showed nearly complete silencing, while in *RNAi*::*NtDIRs #10*, the loss of SAR was associated with reduced expression of *NtDIR3*, but wild-type levels of *NTDIR1* and *NtDIR2*. In comparison, *RNAi*::*NtDIRs #11*, which developed SAR, exhibited wild-type levels of *NtDIR3* expression, but reduced levels of *NtDIR1* and *NtDIR2* (Fig. 2, C and D).

The demonstration that AtDIR1 orthologs play a role in activating SAR in tobacco prompted us to investigate whether the loss of SAR in *RNAi*::*NtDIRs #9* is associated with altered MeSA metabolism. Analysis of *NtSAMT1* transcripts in *RNAi*::*NtDIRs #9* plants indicated that they, like *dir1-1* Arabidopsis, exhibit earlier and stronger expression of *NtSAMT1* in both the TMV-infected and the uninoculated systemic tissue during the first four days following primary TMV infection (Fig. 2E). Based on our findings in Arabidopsis and tobacco, we propose the following model (Fig. 3) for systemic signaling of SAR using Arabidopsis as the example: AtBSMT1, AtDIR1, and AtSFD1 activities are necessary in the primary infected tissue. After pathogen infection, AtDIR1 and an AtSFD1-dependent lipid form a complex in the inoculated leaves that is translocated to the distal systemic tissue. SA levels rise in the primary infected tissue, where some is converted to MeSA by AtBSMT1. Although MSE can convert MeSA back to SA, the rising levels of SA inhibit MSE activity, thereby facilitating a buildup of MeSA for translocation to the distal tissue. In the uninoculated systemic tissue, the MSE activity of AtMES1, 2, 4, 7, and 9 converts biologically inactive MeSA to active SA, which then induces SAR (Vlot et al., 2008b). To facilitate SA accumulation and SAR development, the level of AtBSMT1, which sequesters SA by converting it into MeSA, is directly or indirectly suppressed via reduction in its transcription or mRNA stability by the DIR1-lipid derivative complex (Fig. 3).

It was previously demonstrated that treating the lower leaves of tobacco or Arabidopsis with exogenous MeSA induces SAR in the upper, untreated tissue (Park et al., 2007; Liu et al., 2010). If our model is correct, and the SAR defect in *dir1-1* results from an inability to effectively accumulate SA following its conversion from MeSA (due to increased *AtBSMT1* expression), then MeSA-mediated SAR induction should be compromised in both *dir1-1* Arabidopsis and *NtDIR*-silenced tobacco. To test this possibility, MeSA was infiltrated into three lower leaves of *dir1-1*, *RNAi*::*NtDIRs #9* or wild-type plants, and the upper, untreated leaves were then challenged with pathogen 2 d posttreatment in Arabidopsis and 5 d posttreatment in tobacco. Subsequent analysis of bacterial growth or lesion size indicated that wild-type Arabidopsis and tobacco developed SAR in response to treatment with 3.3 or 5 $\mu$M MeSA, respectively. By contrast, MeSA treatment failed to induce SAR in either *dir1-1* or *RNAi*::*NtDIRs #9* plants (Fig. 4).

Although we did not include *sfd1* in our study, previous reports of *sfd1* and *dir1-1* phenotypes provide further support for our model. The SAR-defective phenotype of *sfd1* was shown to correlate with a failure to accumulate SA in the uninoculated systemic tissue, despite accumulation of wild-type SA levels in the inoculated leaves (Nandi et al., 2004). In addition, treatment with the SA analog benzothiadiazole induced SAR against *Psm* in *sfd1* as well as in wild-type plants (Nandi et al., 2004). Similarly, treating *dir1-1* or wild-type plants with the SA analog 2,6-dichloroisonicotinic acid induced SAR to *Pst* (Maldonado et al.,




B

| | wt | | RNAi::NtDIRs # 9 | | wt | | RNAi::NtDIRs #9 | |
|---|---|---|---|---|---|---|---|---|
| 1° treatment | Mock | TMV | Mock | TMV | 0 µM MeSA | 5 µM MeSA | 0 µM MeSA | 5 µM MeSA |
| 2° TMV lesion size (mm ± SD) | 2.42 ± 0.21 | 1.07 ± 0.11 | 2.25 ± 0.20 | 2.16 ± 0.20 | 2.41 ± 0.19 | 1.80 ± 0.18 | 2.26 ± 0.24 | 2.19 ± 0.16 |
| Reduction (%) 2° lesion size to Mock | N/A | 56 | N/A | 4 | N/A | 25 | N/A | 3 |
| SAR | N/A | + | N/A | - | N/A | + | N/A | - |




**Figure 4.** Exogenous MeSA induced SAR in wild-type Ws-2, but not in *Atdir1-1* or in *NtDIR*-silenced tobacco. A, Three days prior to infection, 0, 3.3, and 6.6 $\mu$M MeSA were infiltrated into lower leaves of wild-type Ws-2 and *Atdir1-1* plants to induced resistance to subsequent challenge by *Pst*. As a positive control, SAR was induced in one set of plants by infection with *Psm AvrRpt2 cor*$^-$. Asterisks indicate statistically significant differences (* = $P < 0.05$, ** = $P < 0.01$, Student's *t* test) in *Pst* growth between untreated (or mock-infected) plants versus MeSA-treated (or *Psm AvrRpt2 cor*$^-$ infected) plants. B, MeSA (0 or 5 $\mu$M) was infiltrated into lower leaves of wild-type or *RNAi*::*NtDIRs #9* tobacco; 5 d posttreatment upper, untreated leaves were inoculated with TMV. Lesion sizes were measured 5 d postinfection (dpi). Pictures showing representative TMV lesions were taken 5 dpi. Tobacco plants induced for SAR via a primary infection with TMV served as a positive control. Two independent experiments were analyzed with similar results in A and B.

2002). The ability of 2,6-dichloroisonicotinic acid or benzothiadiazole to induce SAR in *dir1-1* or *sfd1*, respectively, suggests that DIR1 and SFD1 function upstream of SA accumulation in the systemic tissue, in agreement with our model (Fig. 3). Note that in contrast to SA, these two analogs cannot be inactivated by AtBSMT1-catalyzed methylation.

Studies of Arabidopsis with reduced *AtBSMT1* expression also support our model for systemic SAR signaling. Characterization of an *Atbsmt1* KO mutant indicated that it exhibits normal levels of local resistance to *Pst* and *Hyaloperonospora arabidopsidis* but fails to accumulate MeSA following pathogen infection. This mutant also fails to accumulate SA or SAG in the uninoculated leaves or develop SAR following infection with *Psm AvrRpt2 cor*$^-$ (Liu et al., 2010). While these findings further argue that MeSA plays a role in SAR signaling in Arabidopsis, Zeier and coworkers have presented results that suggest MeSA production is not essential for SAR development (Attaran et al., 2009). Using similar KO mutants in *AtBSMT1*, they observed that although these mutants were compromised in production of MeSA after infection, they still developed SAR. A number of differences between the experimental design, including the developmental stage of the plants, the type of and the inoculum concentration of the pathogen used to induce SAR, as well as experimental conditions, appear to be responsible for these conflicting results. We have been able to reproduce the Zeier's results, confirming that under certain conditions MeSA is not required for SAR (P.P. Liu, C.C. von Dahl, and D.F. Klessig, unpublished data). Currently, we are deciphering the critical factor (s) that determine whether MeSA is required. The results reported here and the proposed working model are applicable only under conditions in which MeSA is required for SAR.

In summary, we propose that SAR under certain conditions is activated via the interplay between at least two mobile signals, MeSA and a complex formed between AtDIR1 and an AtSFD1-dependent lipid or lipid derivative. Why multiple signals are required for SAR activation is currently unclear. One possibility is that the SAR activation pathway(s) has evolved redundancy, as well as checks and balances, to ensure SAR induction occurs only when it is needed. Although SAR plays a critical role in protecting plants against pathogens, it has a significant fitness cost (van Hulten et al., 2006). Moreover, the use of different combinations of signals may provide greater flexibility to activate SAR under different circumstances/conditions and/or when one of the same signals is needed for another process. Indeed, MeSA has been shown to serve not only during defense signaling, but also during mutualistic interactions, where it functions to attract pollinators or to mediate predator attraction to attacking herbivores (Raguso et al., 1996; Snoeren et al., 2010). Given the likely biological and biochemical complexity of inducing SAR, the requirement for any given SAR signal may depend on a combination of physiological, biological, and environmental conditions.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers JF275846 (NtDIR1), JF275847 (NtDIR2), and JF275848 (NtDIR3).

## Supplemental Data

The following materials are available in the online version of this article.

**Supplemental Figure S1.** The tobacco homologs of AtDIR1.

**Supplemental Figure S2.** Alignment of the full-length cDNAs of *AtDIR1*, *NtDIR1*, *NtDIR2*, and *NtDIR3*.

**Supplemental Materials and Methods S1.** Description of materials and experimental procedures presented in this study.

## ACKNOWLEDGMENTS

We thank D'Maris Dempsey for critically reading the manuscript and Dr. Robin K. Cameron for providing seeds of the Arabidopsis *dir1-1* mutant and its control plant (wild-type Ws-2).

Received December 22, 2010; accepted January 28, 2011; published February 10, 2011.

## LITERATURE CITED

**Attaran E, Zeier TE, Griebel T, Zeier J** (2009) Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in *Arabidopsis*. Plant Cell **21:** 954–971

**Browse J, Somerville CR** (1991) Glycerolipid metabolism, biochemistry and regulation. Annu Rev Plant Physiol Mol Biol **42:** 467–506

**Chaturvedi R, Krothapalli K, Makandar R, Nandi A, Sparks AA, Roth MR, Welti R, Shah J** (2008) Plastid omega3-fatty acid desaturase-dependent accumulation of a systemic acquired resistance inducing activity in petiole exudates of *Arabidopsis thaliana* is independent of jasmonic acid. Plant J **54:** 106–117

**Jung HW, Tschaplinski TJ, Wang L, Glazebrook J, Greenberg JT** (2009) Priming in systemic plant immunity. Science **324:** 89–91

**Kachroo A, Venugopal SC, Lapchyk L, Falcone D, Hildebrand D, Kachroo P** (2004) Oleic acid levels regulated by glycerolipid metabolism modulate defense gene expression in Arabidopsis. Proc Natl Acad Sci USA **101:** 5152–5157

**Kachroo P, Shanklin J, Shah J, Whittle EJ, Klessig DF** (2001) A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proc Natl Acad Sci USA **98:** 9448–9453

**Liu PP, Yang Y, Pichersky E, Klessig DF** (2010) Altering expression of benzoic acid/salicylic acid carboxyl methyltransferase 1 compromises systemic acquired resistance and PAMP-triggered immunity in Arabidopsis. Mol Plant Microbe Interact **23:** 82–90

**Maldonado AM, Doerner P, Dixon RA, Lamb CJ, Cameron RK** (2002) A putative lipid transfer protein involved in systemic resistance signalling in Arabidopsis. Nature **419:** 399–403

**Manosalva PM, Park SW, Forouhar F, Tong LA, Fry WE, Klessig DF** (2010) Methyl esterase 1 (StMES1) is required for systemic acquired resistance in potato. Mol Plant Microbe Interact **23:** 1151–1163

**Nandi A, Krothapalli K, Buseman CM, Li MY, Welti R, Enyedi A, Shah J** (2003) *Arabidopsis* sfd mutants affect plastidic lipid composition and suppress dwarfing, cell death, and the enhanced disease resistance phenotypes resulting from the deficiency of a fatty acid desaturase. Plant Cell **15:** 2383–2398

**Nandi A, Welti R, Shah J** (2004) The *Arabidopsis thaliana* dihydroxyacetone phosphate reductase gene SUPPRESSSOR OF FATTY ACID DESATURASE DEFICIENCY1 is required for glycerolipid metabolism and for the activation of systemic acquired resistance. Plant Cell **16:** 465–477

**Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF** (2007) Methyl

salicylate is a critical mobile signal for plant systemic acquired resistance. Science **318:** 113–116

**Park SW, Liu PP, Forouhar F, Vlot AC, Tong L, Tietjen K, Klessig DF** (2009) Use of a synthetic salicylic acid analog to investigate the roles of methyl salicylate and its esterases in plant disease resistance. J Biol Chem **284:** 7307–7317

**Raguso RA, Light DM, Pickersky E** (1996) Electroantennogram responses of Hyles lineata (Sphingidae: Lepidoptera) to volatile compounds from Clarkia breweri (Onagraceae) and other moth-pollinated flowers. J Chem Ecol **22:** 1735–1766

**Seskar M, Shulaev V, Raskin I** (1998) Endogenous methyl salicylate in pathogen-inoculated tobacco plants. Plant Physiol **116:** 387–392

**Shah J** (2009) Plants under attack: systemic signals in defence. Curr Opin Plant Biol **12:** 459–464

**Snoeren TAL, Mumm R, Poelman EH, Yang Y, Pichersky E, Dicke M** (2010) The herbivore-induced plant volatile methyl salicylate negatively affects attraction of the parasitoid *Diadegma semiclausum*. J Chem Ecol **36:** 479–489

**Truman W, Bennett MH, Kubigsteltig I, Turnbull C, Grant M** (2007) Arabidopsis systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. Proc Natl Acad Sci USA **104:** 1075–1080

**van Hulten M, Pelser M, van Loon LC, Pieterse CM, Ton J** (2006) Costs and benefits of priming for defense in Arabidopsis. Proc Natl Acad Sci USA **103:** 5602–5607

**Vernooij B, Friedrich L, Morse A, Reist R, Kolditz-Jawhar R, Ward E, Uknes S, Kessmann H, Ryals J** (1994) Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but it is required in signal transduction. Plant Cell **6:** 959–965

**Vlot AC, Klessig DF, Park SW** (2008a) Systemic acquired resistance: the elusive signal(s). Curr Opin Plant Biol **11:** 436–442

**Vlot AC, Liu PP, Cameron RK, Park SW, Yang Y, Kumar D, Zhou FS, Padukkavidana T, Gustafsson C, Pichersky E, et al** (2008b) Identification of likely orthologs of tobacco salicylic acid-binding protein 2 and their role in systemic acquired resistance in *Arabidopsis thaliana*. Plant J **56:** 445–456

**Yalpani N, Silverman P, Wilson TMA, Kleier DA, Raskin I** (1991) Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. Plant Cell **3:** 809–818