Arabidopsis systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates

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In the absence of adaptive immunity displayed by animals, plants respond locally to biotic challenge via inducible basal defense networks activated through recognition and response to conserved pathogen-associated molecular patterns. In addition, immunity can be induced in tissues remote from infection sites by systemic acquired resistance (SAR), initiated after gene-for-gene recognition between plant resistance proteins and microbial effectors. The nature of the mobile signal and remotely activated networks responsible for establishing SAR remain unclear. Salicylic acid (SA) participates in the local and systemic response, but SAR does not require long-distance translocation of SA. Here, we show that, despite the absence of pathogen-associated molecular pattern contact, systemically responding leaves rapidly activate a SAR transcriptional signature with strong similarity to local basal defense. We present several lines of evidence that suggest jasmonates are central to systemic defense, possibly acting as the initiating signal for classic SAR. Jasmonic acid (JA), but not SA, rapidly accumulates in phloem exudates of leaves challenged with an avirulent strain of *Pseudomonas syringae*. In systemically responding leaves, transcripts associated with jasmonate biosynthesis are up-regulated within 4 h, and JA increases transiently. SAR can be mimicked by foliar JA application and is abrogated in mutants impaired in jasmonate synthesis or response. We conclude that jasmonate signaling appears to mediate long-distance information transmission. Moreover, the systemic transcriptional response shares extraordinary overlap with local herbivory and wounding responses, indicating that jasmonates may be pivotal to an evolutionarily conserved signaling network that decodes multiple abiotic and biotic stress signals.

Arabidopsis thaliana | jasmonic acid | microarray | *Pseudomonas syringae*

Plants use an extraordinarily complex network of synergistic defensive strategies, collectively termed basal defense or nonhost resistance, to protect themselves from diverse pathogens. Fundamental to inducible basal resistance is the ability to recognize and respond to pathogen-associated molecular patterns (PAMPs) (1). Successful bacterial infections suppress basal defense and redirect host metabolism for nutrition and growth. This process requires a functional type III secretion system and is achieved through the collective activities of 30–40 type III effector proteins (T3Es) specifically delivered into the plant cell, in combination with other less-well defined virulence factors such as the jasmonic acid (JA) mimic coronatine (2). If the host possesses a disease-resistance protein competent to directly or indirectly recognize T3Es (avirulence gene products), an alternative suite of signaling pathways is activated, resulting in a localized hypersensitive response and containment of the pathogen (3, 4).

Activation of a hypersensitive response is typically accompanied by establishment of systemic immunity to subsequent infection by a range of normally virulent pathogens (5). Once initiated, this systemic acquired resistance (SAR) is broad-spectrum and durable. Effective systemic immunity requires local generation, long-distance translocation, and perception in remote tissues of an inducing signal. Salicylic acid (SA) plays an important role in establishing and maintaining SAR. Although SA is unlikely to be the essential mobile signal for SAR (6), SAR depends on the accumulation of SA in distal leaves, where it induces a change in cellular redox triggering the reduction of oligomeric disulfide-bound NPR1 (nonexpressor of pathogenesis-related genes), a central regulator of SAR. Active NPR1 monomers translocate to the nucleus and interact with members of the TGA transcription factor family (7, 8) as part of the transcriptional reprogramming associated with SAR. Recent studies of *SFD1* (suppressor of fatty acid desaturase) (9) and *DIR1* (defective in induced resistance) (10), which encodes a putative lipid-transfer protein, implicate lipids in systemic signaling, but the identity of the transmitted molecule(s) remains elusive (11).

The RPM1 pathosystem (12) provides an ideal model to dissect signaling pathways because of both the rapidity (≈5 hours after inoculation (hpi) to visible leaf collapse) and SA-independence of the local hypersensitive response. Here, we dissect the timing and nature of early transcriptional events in naïve tissues associated with establishment of systemic immunity after RPM1 recognition. We demonstrate extremely rapid transcriptional reprogramming in systemically responding tissue with a signal signature highly conserved with local responses to herbivory and wounding in addition to sharing secondary metabolism components with late basal defense responses. Our data strongly implicate a role for jasmonates as rapid inductive SAR signals that appear to act ahead of SA-dependent responses in systemic leaves.

Results

Previously, we identified *PIG*s (PAMPs-induced genes) as components of basal defense that accumulate rapidly in early defense responses after challenge of *Arabidopsis thaliana* with either virulent or *hrp* mutants of *Pseudomonas syringae* pv. *tomato* DC3000 (13, 14). Because some components of SAR are genetically shared with basal defense, we hypothesized that *PIG*s may also contribute to broad-spectrum systemic immunity. The expression patterns of

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Abbreviations: DC3000, *Pseudomonas syringae* pv. *tomato* DC3000; *hrpA*, *Pseudomonas syringae* pv. *tomato* DC3000::*hrpA*⁻; hpi, hours after inoculation; JA, jasmonic acid; PAMP, pathogen-associated molecular pattern; SA, salicylic acid; SAR, systemic acquired resistance.

Data deposition: The microarray data generated associated with this manuscript are available at http://affymetrix.arabidopsis.info/narrays (identifier NASCARRAYS-403).

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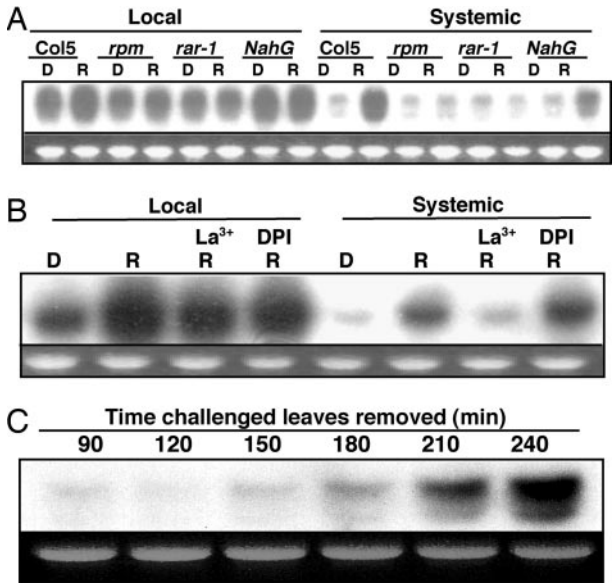

Fig. 1. Rapid systemic induction of *A70* depends on RPM1 recognition. RNA blots of *A70* (At5g56980) accumulation 4 hpi in local or systemic tissues after virulent (D) or avirulent (*avrRpm1*) (R) *P. syringae* pv *tomato* DC3000 challenge. (*A*) *A70* accumulation in RPM1-compromised *rpm1* or *rar1* mutants or in transgenic plants expressing a bacterial salicylic hydroxylase gene (*NahG*) (6). (*B*) Coinfiltration with the calcium channel blocker lanthanum chloride (La^{3+}, 1.5 mM), but not the NADPH oxidase inhibitor diphenyl-iodonium (DPI, 7 μM), blocks systemic *A70* accumulation. (*C*) *A70* induction in systemic leaves 4 hpi with DC3000(*avrRpm1*) after inoculated leaves were removed at the time points indicated.

a subset of *PIG*s were screened by RNA blot against a time course of unchallenged expanding rosette leaves after local challenge of fully expanded leaves with the incompatible DC3000(*avrRpm1*) isolate. We identified one *PIG*, *A70* (At5g56980), rapidly induced in systemic tissue after challenge with DC3000(*avrRpm1*) but not the compatible DC3000 strain (Fig. 1). *A70* induction in inoculated tissues was rapid (≈1 h), sustained, and independent of the pathogen genotype. However, in systemic leaves *A70* transcript accumulated between 3–4 hpi only after challenge with DC3000(*avrRpm1*) but not DC3000 (compatible) (Fig. 1*A*) or DC3000*hrpA*$^-$ (indicative of a basal defense response). Systemic *A70* induction by DC3000(*avrRpm1*) was transient, returning to basal levels by 6 hpi (data not shown). *A70* was not induced in systemically responding leaves of *rpm1* (*rpm1–3*) (12) or *Atrar1* (*Atrar1–28*) (15) mutants, but its induction was near wild type in SA-deficient *NahG* transgenic lines (Fig. 1*A*). Although coinfiltration with calcium ion channel blocker $LaCl_3$ abolished systemic induction of *A70*, neither coinfiltration of the NADPH oxidase inhibitor diphenyl-iodonium nor the NADPH oxidase mutant alleles, *AtrbohD* or *AtrbohF*, (16) affected systemic *A70* expression. Thus, the RPM1-specified increase in $[Ca^{2+}]_{cyt}$ but not the accompanying NADPH oxidase-derived oxidative burst or SA accumulation is necessary for systemic signaling [Fig. 1*B* and data not shown; (17)].

RPM1 recognition triggers specific increases in $[Ca^{2+}]_{cyt}$ at ≈1–1.5 hpi (17), biophotons at ≈2 hpi, and *RPM1*-induced transcripts at ≈3 hpi (13). For systemic *A70* expression, challenged leaves need to be attached to the plant for at least 3 hpi (Fig. 1*C*). Collectively, these data imply rapid local generation, translocation, and subsequent distant decoding of a mobile signal that induces systemic *A70* expression within 1–2 h of RPM1 recognition and before visible collapse of challenged leaves.

We next examined early global gene expression changes in systemic tissues 4 hpi after challenge with either DC3000, DC3000(*avrRpm1*), or DC3000*hrpA*$^-$ and found no significant differences between *hrpA*$^-$ and DC3000 challenges at 4 hpi (see *Materials and Methods*). By contrast, 369 genes were significantly up-regulated and 25 down-regulated after AvrRpm1 delivery [see supporting information (SI) Fig. 6, and SI Table 2].

Secondary Metabolic Pathways Indicative of Plant Basal Defense Are Induced Systemically. Our initial hypothesis predicted that SAR would share similarities with local basal defense. We therefore examined the overlap between the 369 systemically up-regulated genes and genes significantly induced during local basal defense responses (14). Although systemic reprogramming induced fewer transcripts of secondary metabolism pathways associated with basal defense, striking parallels between elements of aromatic amino acid and phenylpropanoid pathways induced in basal defense 12 hpi were already detected systemically at 4 hpi (Fig. 2*A*), whereas, at this time, equivalent local basal responses had not been initiated (14) (SI Fig. 7 and SI Table 3). Notably, *3-deoxy-D-arabino-heptulosonate 7-phosphate synthase* (*DHS1*), whose product controls the entry into aromatic amino acid biosynthesis, and transcripts encoding other key regulatory steps such as phenylalanine ammonia lyase (PAL) are strongly induced, as are phenylpropanoid pathways locally targeted for suppression by type III effector proteins (e.g., 4CL1; SI Table 3) (14). By contrast, key genes whose products specifically control flux and localization of phenylpropanoids on the cell walls are not significantly induced systemically (e.g., cinnamyl alcohol dehydrogenase; Fig. 2*A*). Collectively, these data suggest that the early systemic response primes naïve tissues for rapid recruitment of phenolic-derived basal defense intermediates after secondary infection.

***De Novo* JA Biosynthesis and Induction of Jasmonate-Responsive Genes in Systemic Tissue.** In addition to aromatic amino acid metabolism, almost all of the genes encoding enzymes of the JA biosynthetic pathway (18, 19) were induced systemically, including several of the probe sets with the strongest overall induction (Fig. 2*B*). Many jasmonates, including the JA precursor oxophytodienoic acid (OPDA) and conjugated derivatives, such as methyl-JA or isoleucine-JA have documented roles in defense signaling (20–22). Although the overall oxylipin profile generated systemically may be complex because hydroperoxylyase (HPL1) can lead to formation of the volatiles hexenal and traumatin (23), the strong induction of multiple *OPR* transcripts suggest that oxylipin flux is directed largely toward OPDA and jasmonates. Consistent with this observation, several well characterized JA-responsive transcripts were induced systemically (annotated in SI Table 2), including *VSP2* (*vegetative storage protein*), *CORI1*, and *CORI3* (*coronatine induced*), and, most notably, the bHLH transcription factor *AtMYC2* (*JIN1*; *jasmonate induced*). AtMYC2 activates a subset of transcripts associated with long-distance wound signaling. AtMYC2 also represses some defense-associated genes, including the classical JA markers associated with local pathogen responses, *Thi2.1* and *PDF1.2* (24), neither of which were induced systemically.

To test whether the systemic induction of JA biosynthetic genes led to altered JA levels, the JA content of systemically responding leaves was determined and shown to increase significantly within 6 hpi in DC3000(*avrRpm1*)- but not DC3000*hrpA*-challenged plants (Fig. 3*A*). This JA increase was transient and returned to basal levels by 11 hpi. By contrast, no significant difference in free foliar SA content was found between *avrRpm1*- and *hrp*-challenged plants.

Biological Impact of Jasmonate Signaling Mutants on RPM1 Specified Systemic Immunity. Both transcriptional profiling and targeted metabolite analysis predict an important role for JA in systemic immunity. A70 expression itself is JA- and wound-responsive but is unaffected by heat, cold, or SA treatment (SI Fig. 8). We examined *A70* expression in the jasmonate-insensitive mutant (*sgt1b*/*jai4*) (25,

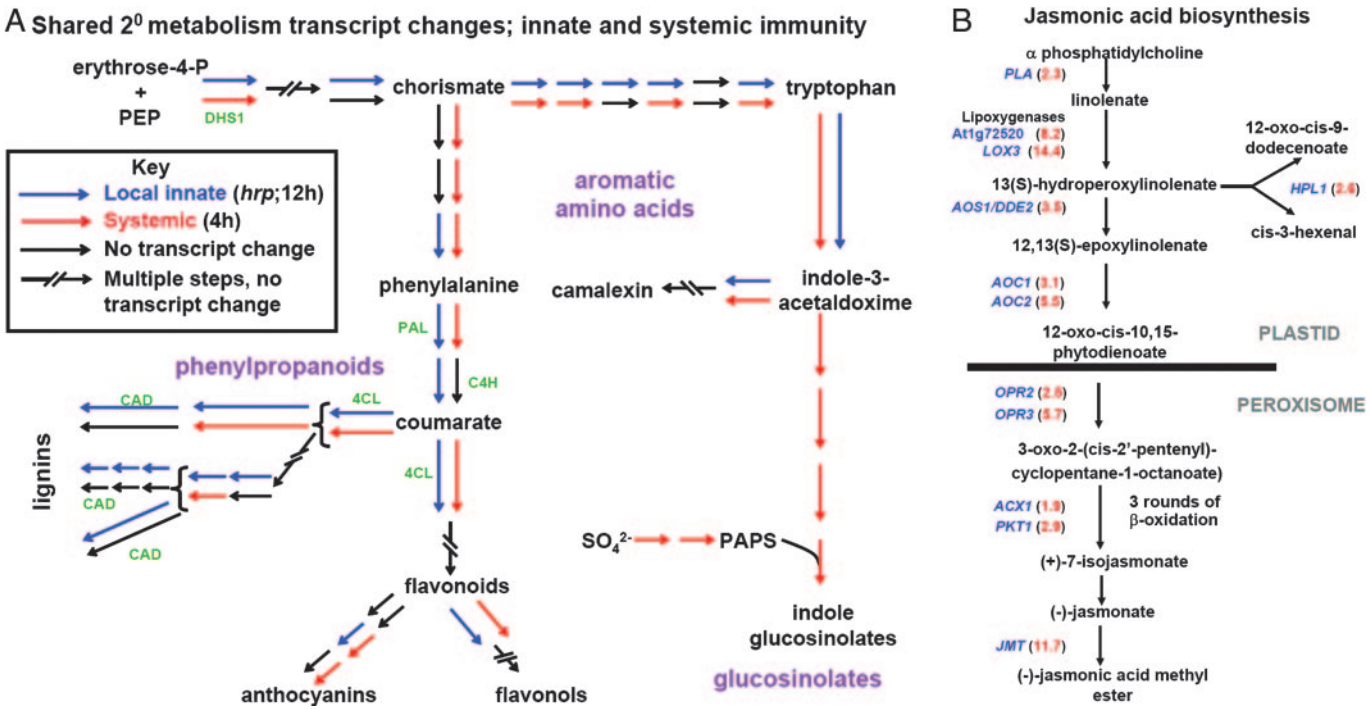

Fig. 2. Overlap of transcriptional reprogramming associated with secondary metabolism in basal and systemic defense. (*A*) Schematic showing commonality and differences in transcriptional induction of secondary metabolism pathways associated with defense. Arrows represent enzymes corresponding to transcripts significantly induced by DC3000*hrpA* (blue arrows), determined 12 hpi, and the systemic transcriptional reprogramming 4 h after local challenge with DC3000(*avrRpm1*) (red arrows). Black arrows represent transcripts with no detectable change. PAL, phenylalanine ammonium lyase; 4CL, 4-coumarate ligase; CAD, cinnamyl alcohol dehydrogenase; C4H, cinnamate-4-hydroxylase. (*B*) Transcriptional induction of the JA biosynthetic pathway. Transcripts encoding JA biosynthesis enzymes were strongly induced systemically at 4 hpi with DC3000(*avrRpm1*). PLA, phospholipase A; AOS, allene oxide synthase; AOC, allene oxide cyclase; OPR, 12-oxophytodienoate reductase 3; PKT, 3-keto-acyl-CoA-thiolase; ACX, fatty acyl-CoA oxidase; JMT, jasmonic acid methyl transferase.

26) and the enhanced disease-susceptible mutant *eds1*, which is a central positive regulator of basal defenses (27), and, based on Fig. 3, a candidate regulator of systemic immunity. Both *sgt1b* and *eds1* modify basal defense and some gene-for-gene responses, but local RPM1 recognition is unaffected. Local and systemic *A70* expression was abolished after DC3000(*avrRpm1*) challenge in *eds1* plants, consistent with a role for *A70* in local basal defenses. In contrast, local *A70* induction was unaffected in *sgt1b* plants, but systemic expression was attenuated or abolished (SI Fig. 9), suggesting that SGT1B may contribute to the perception of the mobile signal responsible for systemic transcriptional reprogramming.

Multiplication of virulent bacteria in wild-type systemic leaves is restricted by ≈40–50 fold 3 days after an immunizing challenge with DC3000(*avrRpm1*). In contrast, systemic immunity in *sgt1b* and *eds1* mutant plants was significantly compromised or completely abrogated, respectively (Fig. 3*B*). As predicted, systemic immunity was also attenuated after DC3000(*avrRpm1*) challenge in the JA biosynthetic mutant *opr3* (28) or the JA response mutant *jin1* (Fig. 3*C*). Conversely, plants sprayed with JA (30 μM) showed restricted virulent bacterial growth (Fig. 3*D*). Collectively, these data strongly support a role for JA in initiation of systemic signaling after DC3000(*avrRpm1*) immunization.

Jasmonate-Mediated Early Systemic Reprogramming Is Conserved. The commonality of systemic reprogramming in other gene-for-gene interactions was investigated by using a combination of biophoton generation (indicative of R protein activation) (29), *A70* expression and reporter genes controlled by either JA biosynthetic [*AOS*::*GUS*; allene oxide synthase (30)] or JA responsive (*VSP2*::*LUC*) promoters. Wild-type leaves challenged with DC3000 carrying *avrRpm1*, *avrRpt2*, or *avrRps4* emit biophotons at ≈2, ≈7, and ≈11 hpi (29) and accumulate *A70* transcript systemically at ≈3, ≈8, and ≈12 hpi, respectively (Fig. 4*A* and data not shown). LUC activity was detected in petioles of unchallenged leaves very soon after local biophoton generation, arising from RPS2 (data not shown) or RPS4 (Fig. 4*B*) recognition and was closely mirrored by GUS activity in *AOS*::*GUS*-expressing lines (Fig. 4*C*). The rapid induction of LUC and GUS activities in petioles after RPS4 recognition and the systemic induction of transcripts encoding JA biosynthetic and response proteins strongly implicate JA in early SAR responses. JA and SA were therefore measured in petiole phloem exudates from DC3000(*avrRpm1*)- or DC3000*hrpA*-challenged leaves. Phloem JA, but not SA, levels of DC3000(*avrRpm1*)-challenged leaves were significantly higher than from leaves inoculated with DC3000*hrpA* (Fig. 4*D*). Although correlative, these data suggest phloem JA export is rapidly enhanced in leaves undergoing an incompatible AvrRpm1/RPM1 reaction, and a JA-based signal may contribute to systemic immunity, before systemic SA accumulation.

Early Systemic Responses Overlap with Local Responses to Wounding, Insect Feeding, or MeJA. The uncovering of a central role for jasmonates in SAR signaling was unexpected. To further understand the nature of the SAR signaling network, the 394 differentially expressed probe sets were used to interrogate experiments representing host responses to biotic and abiotic stresses or hormone treatments at ArrayExpress (www.ebi.ac.uk/arrayexpress/) and NASCArrays (affymetrix.arabidopsis.info) database repositories. Hierarchical clustering tightly associated the systemic response with responses to MeJA application, local wounding, and *Trichoplusia ni* attack (Fig. 5 and SI Fig. 10). The similarities between the early RPM1-dependent systemic response and innate defense responses in infected tissue are illustrated in Table 1 and Fig. 2. A yet

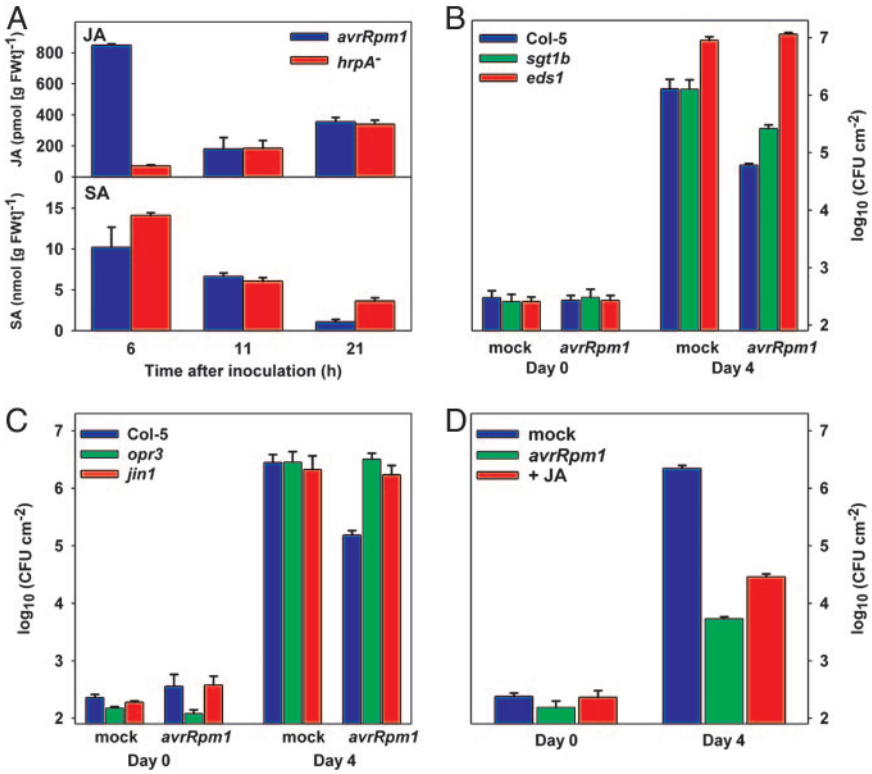

Fig. 3. Competent JA signaling and synthesis are integral to effective SAR. (*A*) Comparison of JA and SA accumulation in systemic leaves of DC3000(*avrRpm1*)- or DC3000*hrpA*-challenged plants. Experiments were repeated three times with similar results. (*B–D*) Multiplication of virulent *P. syringae* 4 days after inoculation in SAR-induced plants. (*B*) SAR is diminished or abolished in the JA response or broad-spectrum defense mutants *sgt1b* and *eds1*, respectively. (*C*) Attenuation of SAR in the JA biosynthetic mutant *opr3* and the JA signaling mutant *jin1*. Experiments were repeated twice with similar results. (*D*) Exogenous JA application (30 μM) restricts virulent bacteria, although not as extensively as DC3000(*avrRpm1*), immunization.

more extensive overlap is evident after MeJA treatment, *T. ni* feeding, or mechanical wounding, collectively accounting for 92% of the systemic response (80% was accounted for by insect feeding alone; Table 1 and Fig. 5), strongly implying that these four responses are elaborated through shared regulatory networks (SI Fig. 10).

Components of Insect Defense Are Induced Systemically. A major transcriptional network distinguishing the local basal defense from systemic responses was the induction of all components of tryptophan biosynthesis and catabolism, ultimately leading to indole glucosinolate biosynthesis, including systemic induction of *UGT74B1*, *sulfotransferase*, (At1g74100) (20, 31), and the key regulatory P450s *CYP79B2*, *CYP79B3*, and *CYP83B1* (SI Table 2). The induction of glucosinolates as an antifeedant defense response is a well characterized JA-mediated signaling process (20, 32) and consistent with establishment of broad-spectrum plant defense. Comparison of glucosinolate profiles demonstrated significant increases in 3-indolylmethylglucosinolate and a trend toward increased 1-methoxy-3-indolylmethylglucosinolate at 18 hpi in sys-

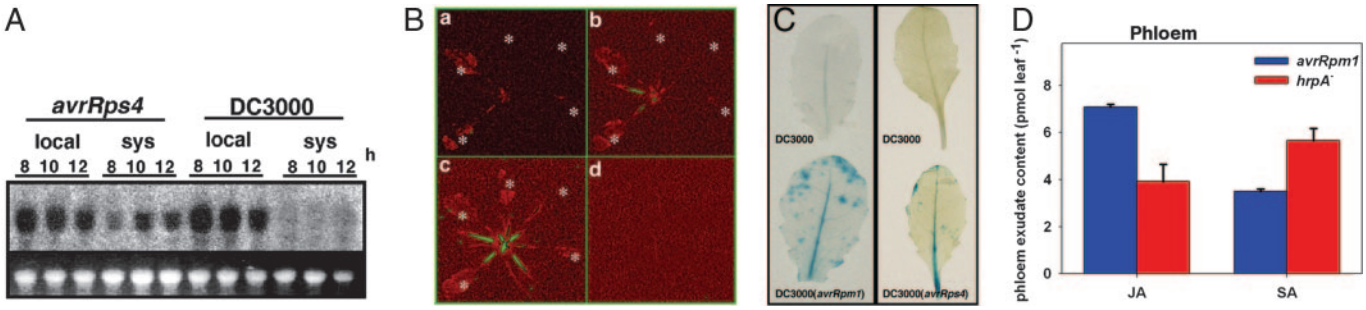

Fig. 4. Evidence for JA as both a mobile and inducing signal in a conserved SAR initiation process. (*A*) RNA blot of *A70* expression after DC3000(*avrRps4*) challenge. (*B*) *VSP2*::*LUC*-expressing plant challenged with DC3000(*avrRps4*) (*a–c*) or DC3000 (*d*). Photons are false colored from red (low emission) to green (high emission). Biophotons are detected only in challenged leaves (asterisks) following RPS4 recognition. Systemic induction of the LUC reporter in the petioles and vegetative meristem occurs rapidly after local biophoton generation. (*a*) Biophoton generation in challenged leaves (asterisk; 12.5–13.25 hpi). (*b*) Biophoton generation and LUC activity in petioles 13.75–14 hpi. (*c*) LUC activity in petioles of challenged and unchallenged leaves, 15–15.75 hpi. (*d*) *VSP2*::*LUC* plant imaged 15–15.75 hpi after DC3000 challenge. (*C*) *AOS*::*GUS* reporter activity is induced within 4 hpi after DC3000(*avrRpm1*) challenge and within 13 hpi after DC3000(*avrRps4*) inoculation. (*D*) JA accumulation in phloem exudates after a local incompatible interaction. SA and JA levels were determined in exudates collected until 5 hpi with DC3000(*avrRpm1*) or DC3000*hrpA*. This experiment was repeated three times with similar results.

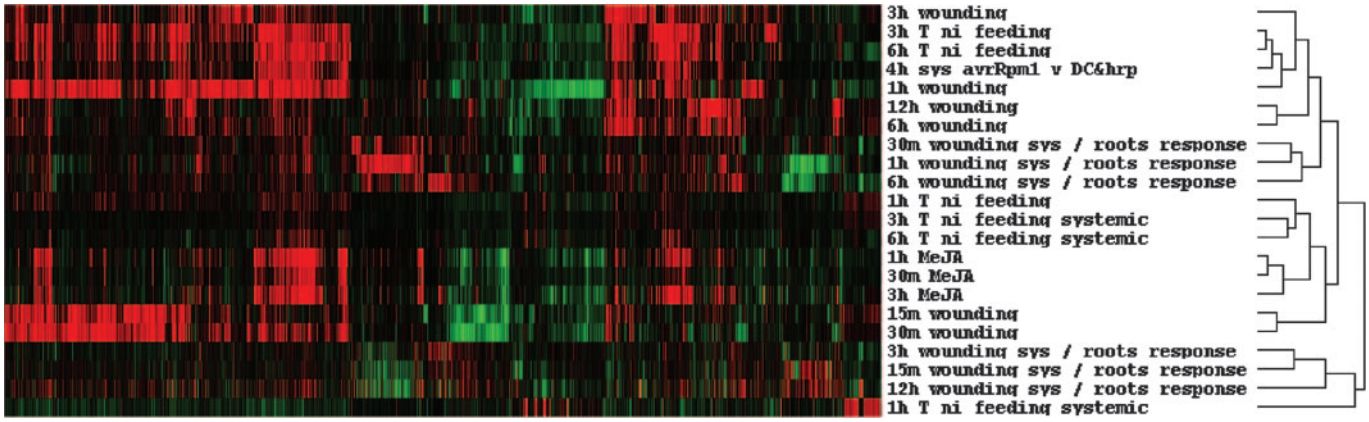

Fig. 5. Overlap of early systemic responses with expression patterns derived from local wounding, insect feeding, and exogenous MeJA application. A combined data set of 2,539 probe sets generated from the systemic response to *avrRpm1* and those displaying a minimum 2-fold change in response to wounding, MeJA treatment, or insect feeding (see SI Fig. 10). These experiments were normalized by using RMA (44), replicate data sets were averaged and set relative to the most obvious control. Genes and experiments were first ordered into self-organizing maps then hierarchically clustered by using complete linkage clustering and an uncentered correlation as the similarity metric.

temic leaves of DC3000(*avrRpm1*)- compared with *hrpA*-challenged plants (SI Table 4).

Discussion

Here, we show that an RPM1-dependent local stimulus generates an unexpectedly rapid mobile signal whose perception in systemically responding tissues leads to (*i*) rapid transcriptional reprogramming, which strikingly mirrors that induced by insect feeding, local wounding, and MeJA application, and (*ii*) *de novo* JA biosynthesis. The JA perception, biosynthetic and signaling mutants, *sgt1b*, *opr3*, and *jin1* all attenuate SAR and corroborate a major role for JA in systemic immunity. Gene-for-gene mediated biophoton generation is rapidly followed by activation of JA-biosynthetic and JA-responsive promoter–reporter fusions, suggesting conservation of systemic signaling across related R proteins. Despite the absence of direct PAMP contact, the RPM1 systemic transcriptional response at 4 hpi shows remarkable similarity to local basal defense at 12 hpi in behavior of components related to aromatic amino acid and phenylpropanoid biosynthesis. In addition, glucosinolate biosynthetic pathway transcripts are induced. Glucosinolates and associated volatiles can act as feeding deterrents (33–35), and glucosinolate-breakdown products can confer enhanced resistance to bacterial pathogens (36).

Although our data do not fit the classic definition of SA-dependent SAR, because RPM1-mediated systemic immunity is attenuated in *npr1* mutants, we propose that the JA and SA phases act in tandem. The characteristics of rapid JA signaling that we have uncovered may represent a previously undetected early initiation phase of systemic resistance, whereas SA contributes to subsequent events in establishment of systemic immunity. Although JA is considered to antagonize SA-dependent responses, the beneficial effects of both signaling pathways need not be mutually exclusive (37) when temporally or spatially separated. Because initial JA increases are transient (Fig. 3*A*) any antagonism toward SA/NPR1-dependent responses may be circumvented by the timing of successive phases of transcriptional regulation. Interestingly, JA is also central to the broad-spectrum induced systemic resistance (ISR) elicited by nonpathogenic rhizobacteria. Although ISR is phenotypically similar to classical SAR, the ISR pathway is SA-independent but responsive to jasmonate and ethylene (38). However, ISR still requires NPR1 function for immunity (39). The parallels of the central role predicted for JA in this study and its role in ISR require further investigation.

Defining the nature of the mobile signal constitutes a major challenge (11). There are intriguing parallels between our results and recent data showing a key role for JA in the tomato systemic wound response. Local JA biosynthesis is necessary after wounding to enable production of a long-distance signal whose recognition in systemic leaves depends on the ability to respond to jasmonate (19, 40). Given that the tobacco lipid transfer protein 1, (LTP1) binds JA (41), and LTP1-JA, but not LTP1 or JA alone, enhances resistance in tobacco to *Phytophthora parasitica* (41), jasmonates are potential ligands for another LTP, DIR1, which mediates systemic signaling (10). With JA-biosynthetic enzymes localized in the sieve elements and companion cells (42), a JA mobile signal could effectively establish systemic immunity through an iterative signaling process involving JA release and uptake along the phloem network, amplifying the systemic signal.

Table 1. Summary of the similarities between the early RPM1-dependent systemic transcriptional response and those associated with local wounding, herbivory, MeJA application, or basal defense

Treatment	Overlap with 369 AvrRpm1-induced genes, *n* (%)
Wounded seedlings 0.25 h, 0.5 h, 1 h, 3 h, or 6 h	283 (76.7)
Tricoplusia ni feeding 1 h, 3 h, or 6 h	296 (80.2)
MeJA application 30 min, 1 h, or 3 h	250 (67.7)
Pst DC3000::*hrpA*$^-$ infection 2 h, 4 h, or 12 h	130 (35.2)

Materials and Methods

Maintenance of Plants and Bacteria. All bacterial strains were grown, cultured, and maintained as described (13). *A. thaliana* plants were grown under short-day conditions as described (13).

Bacterial Inoculations. For RNA, metabolite- and reporter-profiling leaves were inoculated with a 1-ml needleless syringe on their abaxial surface with a bacterial suspension adjusted to OD_{600} 0.2 ($\approx 2 \times 10^8$ cfu·ml^{-1}) in 10 mM $MgCl_2$. For measuring SAR bacterial growth, initial challenges were either mock (10 mM $MgCl_2$) inoculation or challenge with DC3000(*avrRpm1*) at 1×10^5 cfu·ml^{-1}. After 2 days, secondary leaves were infiltrated with either *P. syringae* pv. *maculicola* M4 at 5×10^5 cfu·ml^{-1} or DC3000 at 5×10^4 cfu·ml^{-1}. Bacterial growth titers were determined 3 or 4 days later as described (43).

Chemicals. Stock JA (Sigma, St. Louis, MO) was made to 0.1M in *N-N-* dimethylformamide and diluted with 0.02% Silwet to a final concentration of 50 μM. Stock DPI (ICN, Irvine, CA) was made to 25 mM in dimethylsulfoxide.

Systemic Tissue for Microarrays. For each treatment, a minimum of five fully expanded leaves on 16 plants were infiltrated at 2×10^8 cfu·ml^{-1}. Systemic leaves, comprising all developing leaves up to the half-expanded stage, were harvested at 4 hpi and immediately frozen in liquid nitrogen.

RNA Extraction. Total leaf RNA was isolated as described (13) and subsequently purified by using RNeasy columns (Qiagen, Valencia, CA). All cRNA preparation, labeling, hybridization, and data acquisition of ATH1–121501 Affymetrix GeneChips were performed at the Genomics Centre, John Innes Institute, Norwich, U.K. The complete data set can be found at http://affymetrix.arabidopsis.info/narrays under identifier NASCARRAYS-403.

Normalization and Data Analysis. Three methods were used to normalize the raw data. Robust Multiarray Average [RMA; (44)] by using quantile normalization and the PM-only model-based expression method of dChip (45) were used to analyze the raw Affymetrix signals. Affymetrix GeneChip Microarray Suite version 5.0 software (MAS5.0) was used to obtain signal values scaled to a global intensity of 100.

Significance Analysis of Microarrays (SAM) (46) was conducted on the three independently normalized data sets to identify differentially expressed genes by using a false-discovery rate of 5%. A 1.5-fold expression cutoff was applied for MAS5.0 and dChip-normalized data, and a 1.75-fold change in expression was applied to the RMA-normalized data set (47). Genes with a MAS5.0 signal <50 were excluded, and only those found to be significant with all three normalization methods and passing the absolute expression and minimum fold-change requirements were used for further analyses.

Data Mining. Hierarchical clustering was performed by using CLUSTER (48), visualized with the program TREEVIEW (48). Complete linkage using an uncentered Pearson correlation was applied to RMA-normalized logged data, ordered by self-organizing maps.

ArrayExpress (www.ebi.ac.uk/arrayexpress/) and NASCArrays (affymetrix.arabidopsis.info) data mining used MAS5.0 globally scaled data derived from averaging replicated chips and setting them relative to control chips.

Verification of GeneChip Expression Data. GeneChip expression data were verified by using RNA blots and quantitative RT-PCR (Qiagen). In all instances, the RT-PCR expression levels (for 17 individual genes) corroborated the GeneChip expression data, although generally the fold changes were slightly greater than on the arrays (data not shown).

Phloem Exudates. Infiltrated leaves were removed within 60 min of challenge, and phloem exudates were collected up to 5 hpi into 10 mM K-EDTA (pH 8.0). All experiments were undertaken in triplicate, and the experiments were repeated three times.

Real-Time Imaging. Plants expressing firefly luciferase under control of the *VSP2* promoter were sprayed with luciferin (2 mM in 0.01% Triton X-100, Promega, Madison, WI) 2 h before bacterial inoculation. Challenged plants were placed inside a dark box, and digital Monochrome images were captured on an ORCAII ER CCD camera (Hamamatsu, Hamamatsu City, Japan) with a 35 mm f2.8 Nikkor lens after photon counting for 15 min at 2×2 binning mode and acquisition using Wasabi imaging software (Hamamatsu).

Hormone Measurements. JA and SA content of leaves and phloem were measured by standard methanol extraction and C-18 solid-phase purification as detailed in *SI Text*.

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