Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato

Lei Li*†, Chuanyou Li*†, Gyu In Lee*, and Gregg A. Howe*‡§

Departments of *Energy-Plant Research Laboratory and ‡Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824

Communicated by Clarence A. Ryan, Jr., Washington State University, Pullman, WA, and January 6, 2002 (received for review December 11, 2001)

Plant defense responses to wounding and herbivore attack are regulated by signal transduction pathways that operate both at the site of wounding and in undamaged distal leaves. Genetic analysis in tomato indicates that systemin and its precursor protein, prosystemin, are upstream components of a wound-induced, intercellular signaling pathway that involves both the biosynthesis and action of jasmonic acid (JA). To examine the role of JA in systemic signaling, reciprocal grafting experiments were used to analyze wound-induced expression of the *proteinase inhibitor II* gene in a JA biosynthetic mutant (*spr-2*) and a JA response mutant (*jai-1*). The results showed that *spr-2* plants are defective in the production, but not recognition, of a graft-transmissible wound signal. Conversely, *jai-1* plants are compromised in the recognition of this signal but not its production. It was also determined that a graft-transmissible signal produced in response to ectopic expression of prosystemin in rootstocks was recognized by *spr-2* but not by *jai-1* scions. Taken together, the results show that activation of the jasmonate biosynthetic pathway in response to wounding or (pro)systemin is required for the production of a long-distance signal whose recognition in distal leaves depends on jasmonate signaling. These findings suggest that JA, or a related compound derived from the octadecanoid pathway, may act as a transmissible wound signal.

Lycopersicon esculentum | jasmonic acid | systemin | systemic signaling | induced resistance

Higher plants respond to insect attack and wounding by activating the expression of genes involved in herbivore deterrence, wound healing, and other defense-related processes (1). An important aspect of many induced defense responses is their occurrence in undamaged leaves located distal to the site of attack (2). Wound-inducible proteinase inhibitors (PIs) in tomato (*Lycopersicon esculentum*), which are expressed within ≈2 h after mechanical wounding or herbivory (3, 4), represent one of the best examples of this phenomenon. In their landmark study of wound-inducible PIs, Green and Ryan (2) proposed that specific signals generated at the wound site travel through the plant and activate PI expression in undamaged responding leaves. Although several chemical and physical signals have since been implicated in the systemic wound response (reviewed in refs. 3 and 5–9), very little is known about how these signals interact with one another to effect cell-to-cell communication over long distances.

Among the proposed intercellular signals for wound-induced *PI* gene expression are systemin, an 18-aa peptide derived from proteolytic cleavage of a larger precursor protein called prosystemin (10, 11), and jasmonate signals such as jasmonic acid (JA) and its methyl ester, methyl-JA (MeJA; 12, 13). According to a recent model of wound signaling in tomato (3), systemin is transported through the plant as a mobile signal after its proteolytic release from prosystemin. Interaction of systemin with a plasma membrane-bound receptor (14, 15) then triggers a signaling cascade leading to activation of a lipase that releases linolenic acid from membrane lipids (16, 17). Jasmonates are synthesized from linolenic acid via the octadecanoid pathway and are considered to be key regulators for stress-induced gene expression in virtually all plants (18, 19). Recent studies have shown that 12-oxo-phytodienoic acid, a cyclopentenone precursor of JA/MeJA, is a signal for defense gene expression without its previous conversion to JA (20). Activation of *PI* expression in response to wounding, systemin, and jasmonates involves the coordinate biosynthesis and action of ethylene (21, 22) and is also associated with the production of reactive oxygen species that act downstream of JA (23).

Of relevance to the mechanism of wound-induced intercellular signaling is the observation that genes encoding prosystemin and some JA biosynthetic enzymes are expressed in vascular bundle cells, whereas defensive *PI* genes are expressed in adjacent palisade and spongy mesophyll cells (24–27). The cell-type-specific expression pattern of these signaling components has led to the hypothesis that wound-induced release of systemin into the vascular system activates JA biosynthesis in surrounding vascular tissues in which JA biosynthetic enzymes are located (3). Active transfer or diffusion of a jasmonate signal from its site of synthesis could, in turn, induce *PI* expression in neighboring mesophyll cells. A role for jasmonates in intercellular signaling is supported by the fact that application of JA/MeJA to one leaf induces *PI* expression in distal untreated leaves (13), and that exogenous JA is readily transported in the phloem (28). In addition, it has been demonstrated that cultured plant cells secrete JA into the medium (29). Recent studies suggest that the conversion of JA to MeJA by a specific JA carboxyl methyltransferase is an important regulatory step in jasmonate-mediated intercellular signaling (30).

We are using tomato as a model system for genetic analysis of systemic wound signaling and its role in plant defense. Toward this goal, plant genotypes defective in wound-induced systemic expression of *PI* and other defense-related genes have been identified in various genetic screens (31–33). These mutants can be classified into two phenotypic groups: jasmonate biosynthesis mutants that are insensitive to systemin but responsive to JA/MeJA and jasmonate response mutants that are insensitive to both systemin and JA/MeJA (33). Here we report the use of grafting experiments to determine whether these mutants are defective in the production of a long-distance wound signal, or the recognition of that signal in distal undamaged leaves. The results reveal distinct roles for jasmonate biosynthesis and signaling in the generation and recognition, respectively, of a long-distance wound signal for activation of defense gene expression.

Materials and Methods

Plant Material and Treatments. *Lycopersicon esculentum* cv. Castlemart was used as the wild-type (WT) variety for all experi-

Abbreviations: JA, jasmonic acid; MeJA, methyl JA; WT, wild type; *35S::prosys*, *35S::prosystemin*; PI, proteinase inhibitor; PI-II, proteinase inhibitor II; DHJA, dihydrojasmonic acid.

†L.L. and C.L. contributed equally to this work.

§To whom reprint requests should be addressed. E-mail: howeg@msu.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

 www.pnas.org/cgi/doi/10.1073/pnas.072072599

ments. Plants were grown and maintained as described (4). The original *spr-2* and *jai-1*^{2} mutants (32, 33) were backcrossed to WT, and homozygous mutants lacking the *35S*::*prosystemin* (*35S*::*prosys*) transgene were selected from the resulting F_2 population. Seed for the *spr-2* mutant was collected from a *spr-2*/*spr-2* homozygote that had been back-crossed three times to WT. Because of the reduced fertility of *jai-1*^{2} plants (33), plants homozygous for *jai-1*^{2} were obtained from a segregating F_2 population by using the screening procedure described by Li *et al.* (33). Seed for the *35S*::*prosys* transgenic line was collected from a *35S*::*prosys*/*35S*::*prosys* homozygote that had been back-crossed five times to WT. Wounding was performed as described in the figure legends. Treatment of plants with MeJA (Bedoukian Research, Danbury, CT) was performed as described (34).

Grafting Experiments. Plants (4 weeks old) were grafted by using a modification of the procedure described by McGurl *et al.* (35). A longitudinal incision of ≈1.5 cm was made in the middle of the rootstock stem. The scion stem was trimmed to the shape of a wedge and then tightly fastened to the cortex flaps of the rootstock by using water-soaked raffia. All but one leaf immediately beneath the apical meristem were excised from the scion. Scions were enclosed in a plastic bag that was fastened at the graft junction. After grafting (1 week), the plastic bag and raffia were removed. Grafted plants contained two or three leaves on the rootstock and two newly emerging leaves on the scion. Plants were subjected to mechanical wounding as follows, 4 d after removal of the bag. All leaflets (≈10) on leaves of the stock were crushed with a hemostat across the midvein. This procedure was repeated 3 h later, such that the second wound was parallel to the first wound and proximal to the petiole. Eight hours after the second wound, wounded leaflets from the stock and undamaged leaflets of the scion were harvested separately for RNA isolation. Equal amounts of leaf tissue from three plants of the same graft combination were pooled before RNA isolation. RNA blot analysis of *proteinase inhibitor II* (*PI-II*) mRNA levels was performed as described (34), by using an *eIF4A* cDNA probe as a loading control. PI-II protein levels in grafted plants were measured by radial immunodiffusion assay (36).

Quantification of JA. Plants containing two fully expanded leaves and an emerging third leaf were wounded with a hemostat on each leaflet of the two expanded leaves. Leaves [10 g fresh weight (FW)] were harvested for extraction and quantification of JA by using a modification of the procedure described by Weber *et al.* (37). Harvested leaves were frozen in liquid nitrogen and ground to a fine powder by using a chilled mortar and pestle. The tissue was dissolved in 28 ml of methanol containing 500 ng of dihydrojasmonic acid (DHJA) as an internal standard and then homogenized with a Polytron for 1 min at 4°C. The homogenate was incubated for 2 h at 4°C with shaking, diluted with 12 ml of ice-cold water, and then centrifuged at 3,500 × *g*. The resulting supernatant was recovered and the pH adjusted to 8.0 with NH_4OH. This solution was passed through a tC_{18}-SepPak cartridge (Waters) [preconditioned with 70% (vol) methanol] and collected in a new tube. The cartridge was washed with 7 ml of 75% (vol) methanol. Eluates from both the sample and the wash steps were combined and adjusted to pH 4.0 with 10% (vol) formic acid. This solution was diluted with 160 ml of ice-cold water and then loaded on a tC_{18}-SepPak column that was prewashed sequentially with methanol, diethylether, methanol, and water. After washing the column with 7 ml of 15% (vol) ethanol and 7 ml of water, the JA fraction was eluted with 10 ml of diethylether. The eluate was partially dried over anhydrous $MgSO_4$ and then dried completely under a stream of nitrogen gas. The dried paste was dissolved in 0.5 ml of methanol and subjected to methylation by the addition of diazomethane in 0.5 ml of diethylether. This mixture was dried under nitrogen gas and resuspended in 20 μl of hexane.

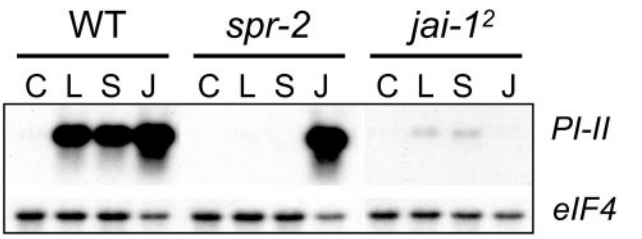

Fig. 1. Induction of the *PI-II* gene in response to wounding and MeJA. Two-leaf stage WT and mutant (*spr-2* and *jai-1*^{2}) tomato plants were wounded once with a hemostat across the midvein of the lower leaf. Leaf tissue was harvested separately (8 hr later) from the wounded leaf (L, local response) and the upper undamaged leaf (S, systemic response) for RNA isolation. RNA was also isolated from a set of plants treated for 12 h with MeJA (J) and a set of untreated control plants (C). Aliquots (5 μg) of total RNA were analyzed by RNA blot analysis for *PI-II* mRNA levels. As a loading control, a duplicate blot was probed with a cDNA encoding *eIF4A*.

The amount of JA/MeJA in leaf extracts was quantified by GC-MS by using a Hewlett-Packard GC 5890 equipped with a Hewlett-Packard 5970 mass detector. The GC was fitted with a DB-5 column and run with a temperature gradient of 100°C for 1 min, 100°C to 170°C at 5°C/min, 170°C for 2.5 min, and 170°C to 250°C at 20°C/min. GC-MS analysis was performed in the SIM mode with monitoring of ions specific for MeJA (m/z = 224) and MeDHJA (m/z = 226). For quantification of JA/MeJA, a standard curve was generated from samples in which MeJA and MeDHJA were mixed in known ratios. Because peaks corresponding to both the 3R,7S and 3R,7R isomers of endogenous JA/MeJA were detected, the areas of the two peaks were combined. JA levels reported in the *Results* section represent the mean ± SE of at least three independent experiments. DHJA was prepared by PtO_2-catalyzed hydrogenation of (±)-JA (Sigma) as described (37). The authenticity of the standard, as well as the absence of endogenous DHJA/MeDHJA in tomato leaf extracts, was verified by GC-MS.

Results

Mutations Affecting Either JA Biosynthesis or JA Signaling Abolish Wound-Induced Systemic Expression of *PI* Genes. Wound response mutants that are defective either in JA biosynthesis or JA responsiveness were used to study the role of JA in systemic wound signaling. The *spr-2* and *jai-1*^{2} mutations were previously identified as suppressors of defense-related responses that are constitutively activated in transgenic tomato plants that overexpress prosystemin from a *35S*::*prosys* transgene (32, 33). *spr-2* plants lack wound-induced systemic expression of the well-characterized *PI-II* gene but nevertheless respond normally to applied MeJA (Fig. 1). This phenotype is very similar to that conditioned by *def-1*, a nonallelic mutation that reduces wound-induced JA accumulation to ≈30% of WT levels (38). To determine whether *spr-2* plants are defective in JA synthesis, JA was extracted from wounded and control (unwounded) leaves of WT and *spr-2* plants and quantified by using GC-MS. The results showed that undamaged WT leaves contained 12 ± 1 pmol JA/g FW. In response to mechanical wounding, JA levels increased to 262 ± 41 and 151 ± 26 pmol JA/g FW 1 h and 3 h, respectively, after wounding. The JA level in unwounded *spr-2* leaves was 3 ± 1 pmol JA/g FW, which rose to 22 ± 9 and 7 ± 1 pmol JA/g FW 1 h and 3 h, respectively, after wounding. This finding indicates that the wound response phenotype of *spr-2* plants results from a defect in jasmonate biosynthesis.

Wounded *jai-1*^{2} plants accumulate *PI-II* mRNA to <5% of the levels observed in WT (Fig. 1). The residual wound-induced expression of *PI-II* in this mutant likely results from partial activity

PLANT BIOLOGY

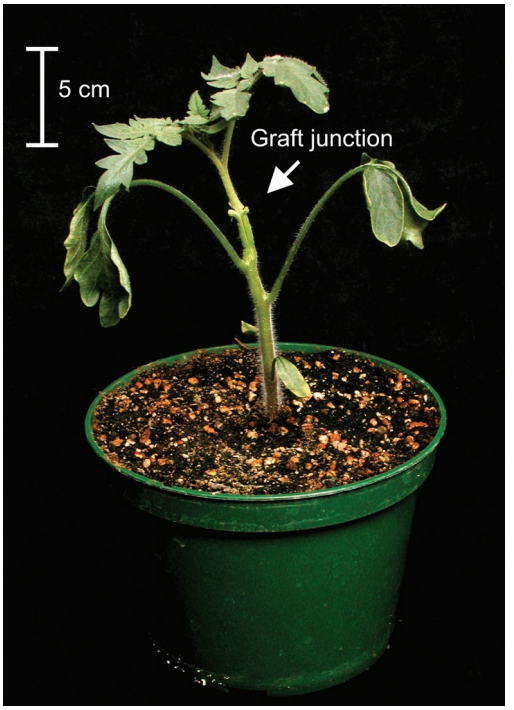

Fig. 2. Photograph of a typical grafted tomato plant. The arrow indicates the position of the graft junction between the stock and scion. Systemic *PI-II* expression was measured in undamaged scion leaves 11 h after wounding of the stock leaves. The distance between wounded leaflets on the stock and undamaged leaflets on the scion is ≈20 cm.

of the *jai-1*^{2} allele, as we have observed that plants homozygous for a deletion allele of *jai-1* (*jai-1*^{1}) accumulate no detectable *PI-II* transcripts in response to wounding (L.L. and G.A.H., unpublished data). In contrast to JA biosynthetic mutants, *jai-1*^{2} plants fail to accumulate *PI-II* transcripts in response to exogenous JA/MeJA (Fig. 1; 33). This phenotype is indicative of a defect in jasmonate perception or subsequent signaling events that are necessary for *PI* activation. Wound-induced JA levels *jai-1* plants were comparable to those in wounded WT plants (G.I.L. and G.A.H., unpublished data).

Jasmonate Signaling Is Required for Functional Recognition of a Long Distance Wound Signal. To examine the role of jasmonate action in the systemic wound response, we analyzed wound-induced *PI-II* expression in grafts between WT and *jai-1*^{2} plants. Four-week-old plants were grafted such that both the rootstock (stock) and the scion contained at least two healthy leaves (Fig. 2). After sufficient time for healing of the graft junction, stock leaves were wounded and *PI-II* mRNA levels were measured 11 h later in both the damaged stock leaves (local response) and the undamaged scion leaves (systemic response). Control experiments showed that the grafting procedure itself induced some *PI-II* expression in WT stock and scion leaves (Fig. 3*A*, lane 1; data not shown). However, subsequent wounding of stock leaves induced local and systemic *PI-II* expression well above this background level (Fig. 3*A*, lane 2). This experiment demonstrates that wounding of WT stock leaves leads to the production of a graft-transmissible signal that is recognized in undamaged scion tissues. Consistent with the wound response observed in *jai-1*^{2} seedlings (Fig. 1), wound-induced expression of *PI-II* in both stock and scion leaves of grafted *jai-1*^{2} plants was <5% of that in WT plants (Fig. 3*A*, lanes 3 and 4). Analysis of *jai-1*/WT hybrid grafts showed that wounding of *jai-1*^{2} stock leaves resulted in full activation of *PI-II* expression in WT scion leaves (Fig. 3*A*, lanes 5 and 6). In the reciprocal combination,

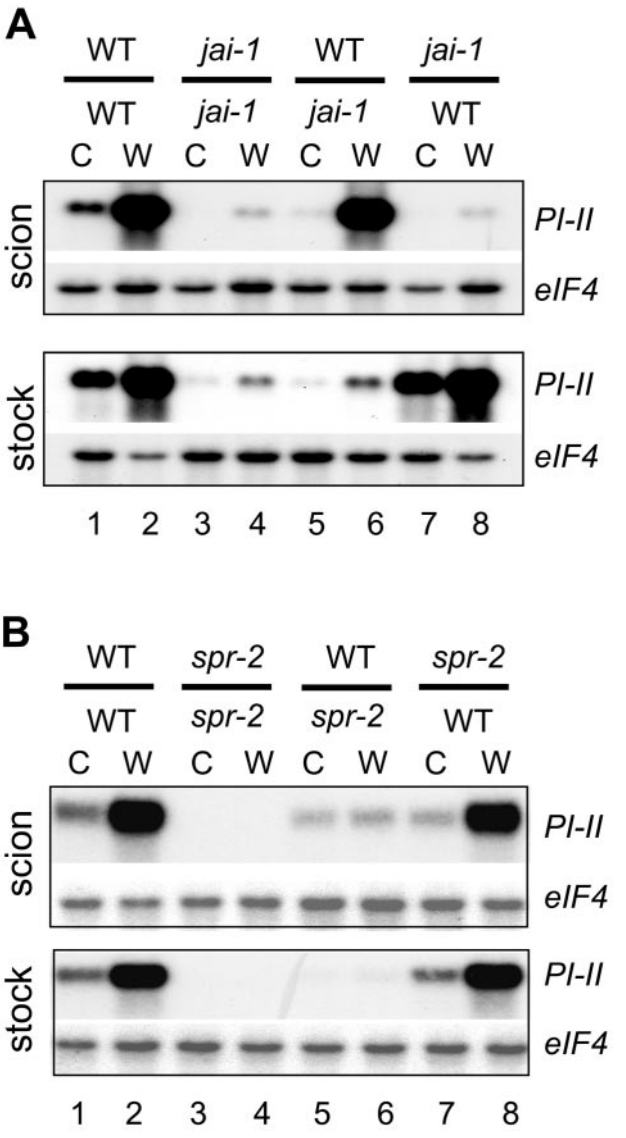

Fig. 3. Wound-inducible *PI-II* expression in grafts between WT plants and mutants defective in jasmonate signaling (*jai-1*) or jasmonate biosynthesis (*spr-2*). WT and *jai-1* plants (*A*) or WT and *spr-2* plants (*B*) were grafted in the four combinations indicated. The genotypes listed above and below the horizontal line correspond to the scion and stock, respectively. For each graft combination, plants were divided into a control (C) and experimental (W) group consisting of three grafted plants per group. For the experimental group, each leaflet on the stock was wounded as described in *Materials and Methods*. Eleven hours after wounding, leaf tissue was harvested separately from wounded stock leaves (stock) and undamaged scion leaves (scion) for RNA extraction. The control set of plants received no wounding, other than that inflicted by the grafting procedure itself. Levels of *PI-II* mRNA were analyzed by RNA blot analysis, using an *eIF4A* cDNA probe as a loading control.

however, undamaged mutant scion leaves failed to express *PI-II* in response to wounding of the WT stock (Fig. 3*A*, lanes 7 and 8). These results demonstrate that *jai-1* does not affect the production of the graft-transmissible systemic signal at the site of wounding but rather disrupts the recognition or proper interpretation of that signal in distal undamaged leaves.

Jasmonate Biosynthesis Is Required for Generation of a Long-Distance Wound Signal. To investigate the role of jasmonate biosynthesis in the systemic wound response, we examined wound-induced *PI-II* expression in graft combinations between WT and *spr-2* plants.

Table 1. PI-II accumulation in WT and *spr-2* scion leaves in response to wounding

Graft combination (Scion/stock)	PI-II in scion, μg/ml of leaf juice	
	Unwounded	Wounded
WT/WT	26 ± 10	117 ± 27
spr-2/*spr-2*	3 ± 2	11 ± 13
WT/*spr-2*	6 ± 1	113 ± 21
spr-2/WT	14 ± 7	17 ± 7

Three plants of each of the indicated graft combinations were constructed from 4-week-old tomato plants. Three weeks after grafting, PI-II levels were measured in two newly developed leaves of the scion, using one excised leaflet per leaf (unwounded). At the same time, 10 of the remaining leaflets on the scion were wounded with a hemostat. PI-II levels in four of the wounded leaflets were determined 48 h later (wounded). Values represent the mean PI-II concentration of three plants per combination ± SD.

In contrast to the results obtained with *jai-1* plants, WT scions showed very little response to wounding of *spr-2* stock leaves (Fig. 3*B*, lanes 5 and 6). However, *spr-2* scions expressed *PI-II* to near WT levels in response to a signal emanating from wounded leaves of WT stock (Fig. 3*B*, lanes 7 and 8). Analysis of grafts between WT plants and the *def-1* mutant, which is also deficient in JA biosynthesis (38), gave results that were similar to those for *spr-2*/WT hybrid grafts (data not shown). These results indicate that JA, or a related octadecanoid pathway-derived compound, is essential for generation of the transmissible wound signal.

The results shown in Fig. 3*B* (lanes 7 and 8) further suggested that recognition of the long-distance signal and subsequent *PI* expression in undamaged *spr-2* scions does not require JA biosynthesis in these tissues. However, an alternative explanation was that grafting of *spr-2* scions to WT stock simply restored the ability of the mutant scion to synthesize JA in response to a signal produced in wounded WT stock leaves. To examine this possibility, *spr-2* scion leaves that had been grafted to WT stock were wounded and then tested for PI-II protein accumulation. Control experiments showed that WT scion leaves grafted to either WT or *spr-2* stock were responsive to wounding (Table 1). *spr-2* scion leaves grafted to *spr-2* stock failed to accumulate PI-II in response to wounding, and this deficiency was not relieved by grafting to WT stock. This finding supports the interpretation that *PI-II* activation in *spr-2* scions (Fig. 3*B*, lanes 7 and 8) does not involve *de novo* JA biosynthesis in these leaves, but rather requires a functional octadecanoid biosynthetic pathway in the WT stock.

Despite the fact that both *spr-2* and *jai-1* abrogate systemic wound signaling, the reciprocal nature of the grafting phenotypes conditioned by each mutation (Fig. 3) predicted that grafted plants lacking both jasmonate responsiveness in stock leaves and jasmonate biosynthesis in scion leaves would be capable of systemic signaling. To test this idea, we examined *PI-II* expression in *spr-2* scion leaves in response to wounding of *jai-1* stock leaves (Fig. 4). The results showed that wounded *jai-1* stock leaves produce a graft-transmissible signal that activates *PI-II* expression in *spr-2* scions (Fig. 4, lanes 7 and 8), at a level comparable to that observed in WT scions (Fig. 4, lanes 3 and 4). As is also predicted from the grafting phenotypes of individual mutants, wound-induced systemic expression of *PI-II* was abolished in grafted plants that are deficient in both JA biosynthesis in stock (i.e., *spr-2*) leaves and JA responsiveness in scion (i.e., *jai-1*) leaves (Fig. 4, lanes 9 and 10).

Roles for Jasmonate Biosynthesis and Signaling in *35S*::*prosys*-Mediated *PI* Expression. Previously it was shown that ectopic expression of prosystemin from a *35S*::*prosys* transgene leads to constitutive *PI* expression in the absence of wounding (35).

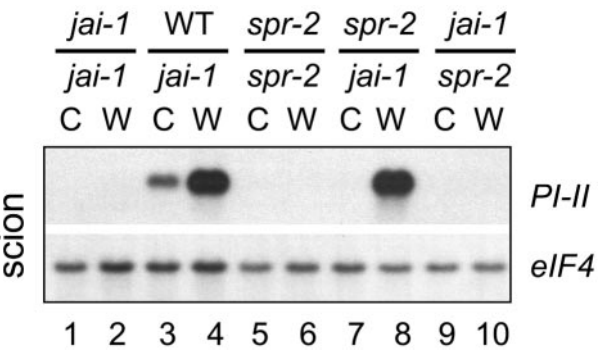

Fig. 4. Wound-induced systemic *PI-II* expression in grafts between *jai-1* and *spr-2* plants. Wound-induced systemic expression of *PI-II* was assessed in the various graft combinations indicated, as described in Fig. 3. *PI-II* and *eIF4* (loading control) mRNA levels in the undamaged scion leaves of unwounded control (C) plants, and plants wounded on the stock leaves (W), are shown.

Grafting experiments presented in the same study further demonstrated that unwounded *35S*::*prosys* stock tissue produces a graft-transmissible signal that activates *PI* expression in WT scion leaves. To investigate the role of jasmonate synthesis and perception in the *35S*::*prosys*-mediated signaling pathway, PI-II protein accumulation was measured in *spr-2* and *jai-1* scions that were grafted to either WT or *35S*::*prosys* stock. As previously reported by McGurl *et al.* (35), WT scion leaves accumulated high levels of PI-II in response to a signal emanating from the *35S*::*prosys* stock (Table 2). The responsiveness of *spr-2* scion leaves to the *35S*::*prosys*-derived signal was comparable to that of WT scions. In contrast, *jai-1* scions were completely unresponsive to the *35S*::*prosys*-derived signal.

Discussion

Systemic activation of defensive *PI* genes in tomato is orchestrated by signaling events that operate both within and between cells. A wealth of biochemical and genetic data support the original proposal (39) that (pro)systemin functions in this pathway to regulate the synthesis of JA, which in turn activates the expression of a subset of target genes including those encoding defensive PIs. However, very little is known about the relationship between systemin-induced JA synthesis and the cell nonautonomous processes by which mobile signals are produced at the wound site, transported through the plant, and perceived by target cells distal to the wound site. To address this question, we used classical grafting techniques

Table 2. PI-II accumulation in grafted tomato plants in response to a long-distance signal generated in a *35S*::*prosystemin* transgenic line

Graft combination (scion/stock)	PI-II, μg/ml of leaf juice	
	Scion	Stock
WT/WT	12 ± 7	18 ± 14
35S::*PS*/*35S*::*PS*	229 ± 14	304 ± 45
WT/*35S*::*PS*	140 ± 36	252 ± 61
spr-2/WT	14 ± 7	16 ± 9
spr-2/*35S*::*PS*	150 ± 12	263 ± 10
jai-1/WT	0	30 ± 11
jai-1/*35S*::*PS*	0	279 ± 11

Tomato plants (5 weeks old) were grafted in the indicated combinations. Three weeks after grafting, PI-II accumulation was measured in three plants of each graft combination. PI-II levels were determined by using three leaflets per leaf. Values represent the mean PI-II concentration of three plants per combination ± SD. *35S*::*PS*, transgenic line that overexpresses prosystemin from a *35S*::*prosystemin* transgene.

PLANT BIOLOGY

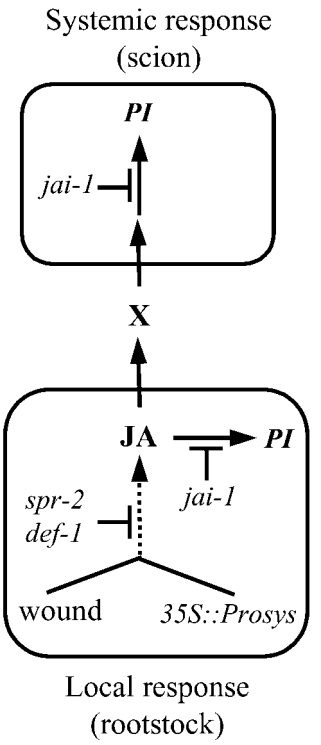

Fig. 5. Genetic model for the role of jasmonate synthesis and signaling in the systemic activation of wound-responsive *PI* genes in tomato plants. Wounding or expression of *35S*::*prosys* leads to the production of a long-distance signal that activates *PI* gene expression in distal leaves (i.e., scion). A functional octadecanoid pathway for jasmonate biosynthesis (hatched line), which is disrupted by *def-1* and *spr-2*, is required for the production of the long-distance signal (X). Jasmonate signaling, which is blocked by *jai-1*, is required for the recognition of the long-distance signal.

to examine long-distance wound signaling in mutants that are deficient either in JA biosynthesis or JA perception. A model consistent with our results and other available genetic data is shown in Fig. 5. This model accounts for the following observations. First, a graft-transmissible signal for systemic *PI* expression is produced in response to either wounding or *35S*::*prosys*. Second, in nongrafted plants, *def-1*, *spr-2*, and *jai-1* suppress both wound- and *35S*::*prosys*-induced *PI* expression. Third, *jai-1* plants are insensitive to both systemin and JA/MeJA, whereas the JA synthesis mutants *spr-2* and *def-1* are insensitive to systemin but responsive to JA/MeJA. Fourth, *jai-1* scions do not respond to a graft-transmissible signal generated either by wounding or *35S*::*prosys*, whereas *spr-2* scions do. Conversely, *spr-2* and *def-1* stock leaves that are deficient in wound-induced JA accumulation are also deficient in wound-induced generation of a graft-transmissible signal, whereas *jai-1* plants that are not impaired in JA biosynthesis are functional in this respect. Taken together, these findings indicate that activation of the octadecanoid pathway in damaged leaves is required for the production of a long-distance signal whose functional recognition in distal leaves requires jasmonate action.

It should be emphasized that other systemic wound responses may operate independently of or in parallel to the systemin/jasmonate pathway that regulates the synthesis of PIs and other defensive phytochemicals. For example, hydraulic signals may be involved in rapid systemic wound responses such as the activation of a wound-inducible protein kinase activity in tomato leaves (7, 40). There is also evidence that genes whose expression in tomato plants is rapidly and systemically induced by wounding are regulated by signaling pathways that operate independently of systemin and JA (4, 8, 41). Such a pathway may account for the residual signaling activity observed in WT scions in response to wounding of *spr-2* (Fig. 3*B*) or *def-1* (data not shown) stock leaves. Alternatively, this residual signaling may reflect incomplete loss of function of *Spr-2*/*Def-1*.

Although systemic activation of *PI* genes clearly involves jasmonate-mediated signaling events in undamaged (scion) responding leaves, grafting experiments conducted with *spr-2* and *def-1* plants indicate that JA biosynthesis is likely not required in these leaves. This observation raises the question of whether *PI* expression in undamaged leaves is mediated by JA or a related octadecanoid signal. Previous studies aimed at addressing this question suggest that JA, rather than C_{18} precursors of JA, is the active signal for *PI* expression in tomato leaves (42). If JA is a signal for *PI* expression in undamaged leaves, our results, together with reports of wound-induced systemic increases in JA levels in tomato (43), suggest that JA is transported from its site of synthesis in stock tissues to undamaged responding leaves. An alternative hypothesis is that the requirement for *Jai-1*-dependent signaling in undamaged leaves is fulfilled by a jasmonate signal other than JA. Candidates for such a signal include 12-oxo-phytodienoic acid and its C_{16} analog, dinor-oxo-phytodienoic acid (37). A more precise understanding of how *def-1* and *spr-2* affect the octadecanoid pathway should provide additional insight into this possibility, as will grafting experiments using transgenic plants that are engineered for a deficiency in specific octadecanoid pathway enzymes.

The roles of jasmonate biosynthesis and perception in wound-induced systemic signaling appear to be similar to their roles in *35S*::*prosys*-mediated *PI* expression (Fig. 5). This conclusion is based on the finding that *spr-2* and *def-1* (L.L. and G.A.H., unpublished data) scions respond to a graft-transmissible signal generated in *35S*::*prosys* stock, whereas *jai-1* scions do not (Table 2). Because *spr-2* and *def-1* plants are insensitive to exogenous systemin (32, 38), it seems unlikely that the graft-transmissible signal produced in *35S*::*prosys* plants is systemin. Rather, our results suggest that this signal is a compound that acts downstream of *Spr-2*/*Def-1* and through *Jai-1*. Possible candidates for this signal include jasmonates and H_2O_2 (23). Measurement of these compounds in *35S*::*prosys* plants may help to address this question. It is interesting to note that although *spr-2* and *def-1* scions respond to the *35S*::*prosys*-derived signal, these mutations effectively suppresses *35S*::*prosys*-mediated signaling when present in homozygous state in the *35S*::*prosys* genetic background (32, 38). This observation indicates that normal *Spr-2* and *Def-1* activity, and thus a functional octadecanoid pathway, is required for production of the *35S*::*prosys*-derived graft-transmissible signal.

The grafting experiments reported herein demonstrate that jasmonate biosynthesis and action, while both required for long-distance activation of *PI* genes, operate at distinct spatial positions along the systemic signaling pathway. More specifically, jasmonate synthesis is required for the generation of the mobile wound signal, whereas jasmonate action is involved in the recognition of this signal in responding leaves. The most straightforward interpretation of these findings is that jasmonate is an essential component of the transmissible wound signal. Moreover, our results are consistent with the hypothesis that JA, MeJA, or a related compound either acts as an intercellular signal (3, 13, 28–30, 44) or triggers the production of such a signal. A central role for jasmonates in systemic wound signaling in tomato plants raises the question of the role of systemin in this response. As discussed previously (3, 13, 27), localized production of systemin at the site of wounding may induce the synthesis of JA/MeJA, which in turn could promote gene expression in neighboring cells. Although this model implies that JA/MeJA act in a paracrine fashion analogous to eicosanoid signals in animal cells, it is conceivable that jasmonates exert their effects over much longer distances. Alternatively, systemin-induced activation of the octadecanoid pathway could further amplify the

signaling cascade through positive feedback on (pro)systemin production or action (15, 25). Identification of mutants that are defective in (pro)systemin perception may provide additional insight into the role of this polypeptide in the systemic wound response, and the mechanism by which it regulates the octadecanoid pathway.

We thank Dr. Douglas Gage in the National Institutes of Health–Michigan State University Mass Spectrometry Facility for helpful assistance with JA measurements. This research was supported by grants from the National Institutes of Health (R01GM57795), the U.S. Department of Energy (DE-FG02-91ER20021), and the Michigan Agriculture Experiment Station at Michigan State University.

1. Karban, R. & Baldwin, I. T. (1997) *Induced Responses to Herbivory* (Chicago Univ. Press, Chicago).
2. Green, T. R. & Ryan, C. A. (1972) *Science* **175,** 776–777.
3. Ryan, C. A. (2000) *Biochim. Biophys. Acta* **1477,** 112–121.
4. Howe, G. A., Lee, G. I., Itoh, A., Li, L. & DeRocher, A. (2000) *Plant Physiol.* **123,** 711–724.
5. Ryan, C. A. (1992) *Plant Mol. Biol.* **19,** 123–133.
6. Bowles, D. J. (1993) *Semin. Cell Biol.* **4,** 103–111.
7. Malone, M. (1996) *Adv. Bot. Res.* **22,** 163–228.
8. Walling, L. L. (2000) *J. Plant Growth Regul.* **19,** 195–216.
9. León, J., Rojo, E. & Sánchez-Serrano, J. J. (2001) *J. Exp. Bot.* **52,** 1–9.
10. Pearce, G., Strydom, D., Johnson, S. & Ryan, C. A. (1991) *Science* **253,** 895–989.
11. McGurl, B., Pearce, G., Orozco-Cárdenas, M. & Ryan, C. A. (1992) *Science* **255,** 1570–1573.
12. Farmer, E. E. & Ryan, C. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7713–7716.
13. Farmer, E. E., Johnson, R. R. & Ryan, C. A. (1992) *Plant Physiol.* **98,** 995–1002.
14. Meindl, T., Boller, T. & Felix, G. (1998) *Plant Cell* **10,** 1561–1570.
15. Scheer, J. M. & Ryan, C. A. (1999) *Plant Cell* **11,** 1525–1535.
16. Narváez-Vásquez, J., Florin-Christensen, J. & Ryan, C. A. (1999) *Plant Cell* **11,** 2249–2260.
17. Ishiguro, S., Kawai-Oda, A., Ueda, J., Nishida, I. & Okada, K. (2001) *Plant Cell* **13,** 2191–2209.
18. Reymond, P., Weber, H., Damond, M. & Farmer, E. E. (2000) *Plant Cell* **12,** 707–720.
19. Schaller, F. (2001) *J. Exp. Bot.* **52,** 11–23.
20. Stintzi, A., Weber, H., Reymond, P., Browse, J. & Farmer, E. E. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 12837–12842.
21. Felix, G. & Boller, T. (1995) *Plant J.* **7,** 381–389.
22. O'Donnell, P. J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H. M. O. & Bowles, D. J. (1996) *Science* **274,** 1914–1917.
23. Orozco-Cárdenas, M., Narváez-Vásquez, J. & Ryan, C. A. (2001) *Plant Cell* **13,** 179–191.
24. Shumway, L. K., Rancour, J. M. & Ryan, C. A. (1970) *Planta* **93,** 1–14.
25. Jacinto, T., McGurl, B., Franceschi, V., Delano-Freier, J. & Ryan, C. A. (1997) *Planta* **203,** 406–412.
26. Kubigsteltig, I., Laudert, D. & Weiler, E. W. (1999) *Planta* **208,** 463–471.
27. Hause, B., Stenzel, I., Miersch, O., Maucher, H., Kramell, R., Ziegler, J. & Wasternack, C. (2000) *Plant J.* **24,** 113–126.
28. Zhang, Z.-P. & Baldwin, I. T. (1997) *Planta* **203,** 436–441.
29. Parchmann, S., Gundlach, H. & Mueller, M. J. (1997) *Plant Physiol.* **115,** 1057–1064.
30. Seo, H. S., Song, J. T., Cheong, J. J., Lee, Y. H., Lee, Y. W., Hwang, I., Lee, J. S. & Choi, Y. D. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 4788–4793.
31. Lightner, J., Pearce, G., Ryan, C. A. & Browse, J. (1993) *Mol. Gen. Genet.* **241,** 595–601.
32. Howe, G. A. & Ryan, C. A. (1999) *Genetics* **153,** 1411–1421.
33. Li, L., Li, C. & Howe, G. A. (2001) *Plant Physiol.* **127,** 1414–1417.
34. Li, L. & Howe, G. A. (2001) *Plant Mol. Biol.* **46,** 409–419.
35. McGurl, B., Orozco-Cárdenas, M., Pearce, G. & Ryan, C. A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 9799–9802.
36. Ryan, C. A. (1967) *Anal. Biochem.* **19,** 434–440.
37. Weber, H., Vick, B. A. & Farmer, E. E. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 10473–10478.
38. Howe, G. A., Lightner, J., Browse, J. & Ryan, C. A. (1996) *Plant Cell* **8,** 2067–2077.
39. Farmer, E. E. & Ryan, C. A. (1992) *Plant Cell* **4,** 129–134.
40. Stratmann, J. W. & Ryan, C. A. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 11085–11089.
41. O'Donnell, P. J., Truesdale, M. R., Calvert, C. M., Dorans, A., Roberts, M. R. & Bowles, D. J. (1998) *Plant J.* **14,** 137–142.
42. Wasternack, C., Ortel, B., Miersch, O., Kramell, R., Beale, M., Greulich, F., Feussner, I., Hause, B., Krumm, T., Boland, W., *et al.* (1998) *J. Plant Physiol.* **152,** 345–352.
43. Herde, O., Atzorn, R., Fisahn, J., Wasternack, C., Willmitzer, L. & Peña-Cortés, H. (1996) *Plant Physiol.* **112,** 853–860.
44. Löbler, M. & Lee, J. (1998) *Trends Plant Sci.* **3,** 8–9.