Endogenous peptide defense signals in *Arabidopsis* differentially amplify signaling for the innate immune response

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***At*Pep1, a 23-aa peptide encoded by *Arabidopsis PROPEP1*, a member of a small, six-member gene family, activates expression of the defense gene *PDF1.2* (encoding defensin) and its own precursor gene, *PROPEP1*, through the jasmonate/ethylene signaling pathway, mediated by a cell-surface receptor, PEPR1. Overexpression of two family members, *PROPEP1* and *PROPEP2*, enhances resistance of *Arabidopsis* plants against the pathogen *Pythium irregulare*, and *PROPEP2* and *PROPEP3* are expressed at highly elevated levels in *Arabidopsis* in response to pathogen infections and to several pathogen-associated molecules (general elicitors). Here, we report that *PDF1.2*, *PR-1* (pathogenesis protein), and *PROPEP* genes were differentially expressed in the leaves of intact plants sprayed with methyl jasmonate and methyl salicylate and in excised leaves supplied through cut petioles with peptides derived from the C terminus of each of the encoded proteins. The expression of *PDF1.2* and *PR-1* elicited by the peptides was blocked in mutant plants deficient in the jasmonate/ethylene and salicylate pathways, and in wild-type plants by treatment with diphenylene iodonium chloride, an inhibitor of hydrogen peroxide production. *PROPEP1*, *PROPEP 2*, and *PROPEP3* genes appear to have roles in a feedback loop that amplifies defense signaling pathways initiated by pathogens.**

defensin | jasmonate | plant defense | PR-1 | salicylic acid

Innate immunity in plants, as in animals, is defined as the receptor-mediated surveillance system that detects the presence of pathogen-associated molecular patterns (PAMPs) and activates defense genes to provide the first line of host defense against infection (1, 2). Only recently have studies of plant innate immunity, especially in *Arabidopsis* (3), provided molecular details that have helped define innate immunity in plants. These and others studies have revealed that although the overall strategies of animals and plants for monitoring the presence of pathogens are strikingly similar, the intracellular signaling pathways are quite different (4–6). In plants, the well known general elicitors that activate plant defense genes are now referred to as PAMPs (2, 6), and they are recognized by leucine-rich repeat (LRR) receptor kinases to initiate intracellular signaling activating the expression of defense genes. We recently reported the isolation of a 23-aa peptide from *Arabidopsis* leaves that is an endogenous elicitor of defensin, which is encoded by *PDF1.2*, a gene associated with innate immunity in plants (7). The peptide is processed from a precursor protein, PROPEP1 (7), whose gene is a member of a small gene family composed of six annotated genes and one unannotated gene. The receptor for *Arabidopsis thaliana* (*At*-)Pep1, PEPR1, has been isolated and identified as an LRR receptor kinase (8). We report herein that the six annotated genes are differentially regulated by methyl jasmonate (MeJA) and methyl salicylate (MeSA) and by peptides derived from the six precursors encoded by the genes. The six peptides also differentially regulate the expression of *PDF1.2*, *PR-1* (which encodes pathogenesis protein 1). The data presented here support our previously proposed role for *PROPEP* genes (7) as endogenous amplifiers of innate immune responses that are initiated by PAMPs.

Results and Discussion

***PROPEP* Gene Family Members in *Arabidopsis* Are Differentially Expressed in Leaves in Response to MeJA and MeSA.** *Arabidopsis PROPEP1* (At5g64900) is expressed in the plant's leaves in response to MeJA and ethylene, but expression of other paralogs of the six-member gene family has not been investigated. In Fig. 1*A*, the expressions of all six paralogs are shown in response to spraying plants with 625 μM MeJA or 2 mM MeSA, and assayed 2 h later. The 2-h time point was chosen from time course analyses in response to both treatments. In response to MeJA, *PROPEP1* and *PROPEP2* (At5g64890) were highly expressed, with *PROPEP4* (At5g09980) being moderately expressed (Fig. 1*A*). *PROPEP3* (At5g64905), *PROPEP5* (At5g09990), and *PROPEP6* (At2g22000) appear to be unaffected by MeJA.

In plants sprayed with 2 mM MeSA, only *PROPEP2* and *PROPEP3* were expressed over basal levels (Fig. 1*B*). These two genes have been shown to be highly expressed in response to fungal, bacterial, and oomycete pathogens, and to elicitors (PAMPs) derived therefrom (7, 9, 10). The high expression of the two genes in response to pathogens and PAMPs and their expression in response to MeJA and MeSA were indicators of their potential importance in the innate immune response. Because it is not known whether any of the genes are tissue-specific or cell-specific, little can be deduced about the causes of the different levels of expression.

***At*Pep Peptides Differentially Regulate Expression of *PROPEP* Gene Family Members.** *PROPEP1* expression in excised *Arabidopsis* leaves is induced by supplying low nanomolar concentrations of *At*Pep1 through cut petioles (7). However, expression of the other five *PROPEP* gene family members by peptides derived from the C terminus of each gene has not been assayed. The peptide from each gene had been synthesized based on their homology to native *At*Pep1 (7) and shown to be active at nanomolar concentrations in the alkalinization assay with *Arabidopsis* suspension-cultured cells. All except *At*Pep4 competed strongly with radiolabeled *At*Pep1 for binding to the receptor PEPR1 (8).

In Fig. 2, the expressions of *PROPEP1* and the other precursor genes, *PROPEP2* (At5g64890), *PROPEP3* (At5g64905), *PROPEP4* (At5g09980), *PROPEP5* (At5g09990), and *PROPEP6*

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Abbreviations: MeJA, methyl jasmonate; MeSA, methyl salicylate; SA, salicylate; JA/Et, jasmonate/ethylene; PDF1.2, defensin; PR, pathogenesis protein; PAMP, pathogen-associated molecular pattern; PEPR1, *At*Pep1 receptor.

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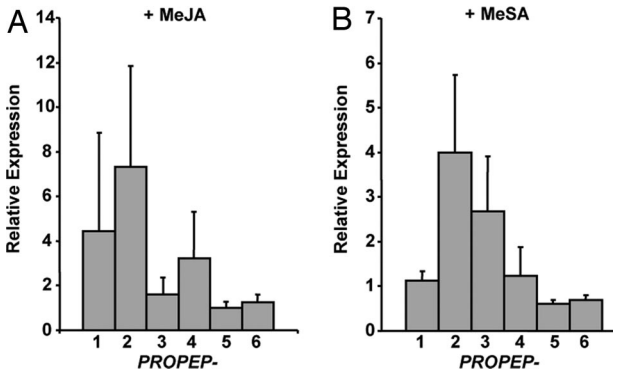

Fig. 1. Inducibility of *PROPEP* family genes by MeJA and MeSA. (*A*) Relative fold change in expression of *PROPEP* family genes in *Arabidopsis* plants sprayed with 625 μM MeJA compared with untreated control plants as determined by semiquantitative RT-PCR analysis using the β-tubulin gene as a control. (*B*) Relative expression of *PROPEP* genes in plants sprayed with 2 mM MeSA versus control plants. Error bars indicate standard deviation from the mean of three experiments.

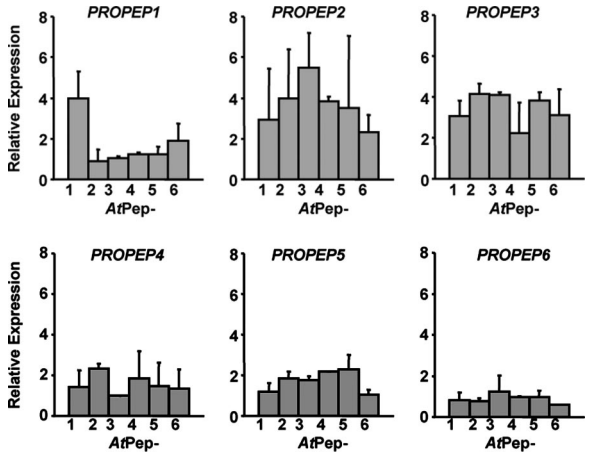

Fig. 2. Expression of *PROPEP* precursor genes in response to *At*Pep peptides. Relative fold change in expression of *PROPEP* family genes in leaves supplied with 20 nM *At*Pep peptides relative to control leaves supplied with water, determined by semiquantitative RT-PCR analysis.

(At2g22000), were assayed by supplying each *At*Pep peptide at 20 nM concentration to excised *Arabidopsis* leaves for 2 h before assaying mRNA levels by RT-PCR. The 2-h time points were selected because excision (wounding) caused increased background expression levels and complicated interpretations of the data at later time points. Only *At*Pep1 strongly induced the expression of *PROPEP1* (Fig. 2*A*). However, *PROPEP2* and *PROPEP3* were strongly induced by all of the peptides (Fig. 2 *B* and *C*), and *PROPEP4* and *PROPEP5* were only weakly expressed (Fig. 2 *D* and *E*). *PROPEP6* was not expressed in response to any peptide (Fig. 2*F*). Why the different peptides have differential activities in inducing the expression of the six *PROPEP* paralogs *in planta* is not understood, but it may be influenced by the composition of receptor complexes that determine intracellular signaling in leaves.

AtPep Peptides Regulate Expression of Pathogen Defense Genes Associated with both Salicylate (SA) and Jasmonate/Ethylene (JA/Et) Signaling Pathways. *At*Pep1 induces the expression of *PDF1.2* in excised wild-type *Arabidopsis* leaves but not in *ein2-1* (ethylene-insensitive) (11) or *fad3,7,8* (jasmonate-deficient) (12) mutants (7). The other five peptides were initially assayed for their ability to induce the expression of *PDF1.2* in wild-type plants, compared with *At*Pep1. *At*Pep2 induced *PDF1.2* expression more strongly than *At*Pep1 (Fig. 3*A*), but the other peptides were only weakly active (Fig. 3*A*). Induction of *PDF1.2* in leaves of *ein2–1* and *fad3,7,8* mutants by each of the peptides is blocked (Fig. 3*A*). These experiments indicate that the expression of *PDF1.2* in leaves by *At*Pep peptides is most strongly induced by *At*Pep1 and *At*Pep2, and the induction by each requires a functional JA/Et pathway. The other peptides weakly induce *PDF1.2* expression, and this induction also appears to require the JA/Et pathway.

The possible effect of *At*Pep peptides on *PR-1* expression had not been assayed previously in *Arabidopsis* plants. The expression of the gene encoding PR-1 has been a model for studies of the SA defense signaling pathway and has been shown to be blocked in mutants including *npr1-1*, a SA signaling pathway mutant (13), and *sid2-2*, a SA biosynthetic mutant (14). In Fig. 3*B* is shown that with the exception of *At*Pep4, the expression of *PR-1* is strongly induced by supplying excised leaves with 20 nM of each of the *At*Pep peptides. *At*Pep4 had previously been shown to be the weakest competitor of the six peptides for binding to the receptor PEPR1 (8). The six peptides were supplied to *Arabidopsis* leaves from *npr1-1* and *sid2-2* mutants to determine whether *PR-1* expression depended on the SA signaling pathway. *PR-1* was poorly

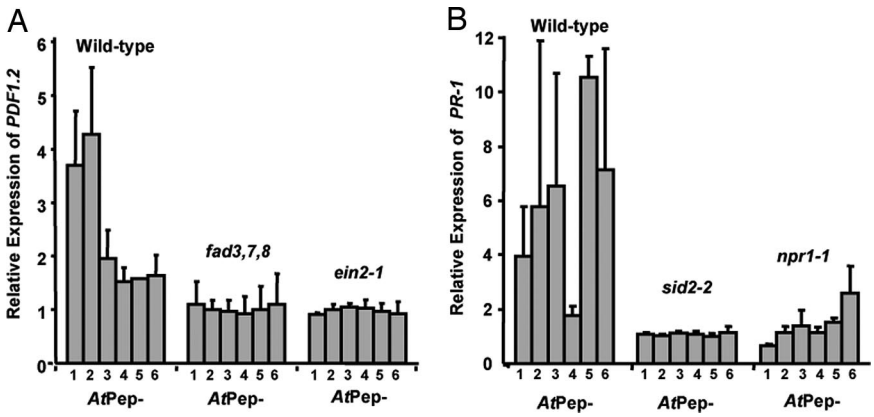

Fig. 3. *At*Pep-induced *PDF1.2* and PR-1 expression is blocked in mutant plants. (*A*) Relative expression of *PDF1.2* in leaves of wild-type *Arabidopsis*, *fad* triple-mutant, and *ein2-1* plants leaves supplied with 20 nM solution of *AtPep* peptides through their cut petioles, compared with control leaves supplied with water. (*B*) Expression of *PR-1* in leaves from wild-type *Arabidopsis*, *sid2-2*, and *npr1-1* mutant plants supplied with 20 nM *At*Pep peptides through their cut petioles relative to leaves supplied with water. Expression of *PDF1.2* and *PR-1* was determined by semiquantitative RT-PCR.

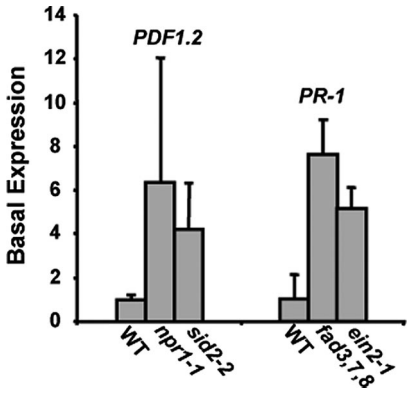

Fig. 4. (*Left*) Basal expression levels of *PDF1.2* in untreated wild-type (WT) *Arabidopsis* plants compared with the basal levels in untreated *npr1-1* and *sid2-2* mutant plants. (*Right*) Basal expression levels of *PR-1* in wild-type plants compared with basal levels in *fad3,7,8* and *ein2-1* mutant plants.

expressed in both mutants in response to all of the peptides, indicating that *PR-1* induction by each peptide depended on a functional SA pathway.

Anomalies were noted in the basal levels of *PDF1.2* and *PR-1* expression compared with wild-type plants. The *sid2-2* and *npr1-1* mutant plants had a strikingly increased basal expression of *PDF1.2*, and the *fad3,7,8* and *ein2-1* mutant plants highly expressed *PR-1* (Fig. 4). These data indicated that there was some type of cross-talk occurring between the two pathways. It appears that when one pathway is blocked, the other pathway may sense a change in the intracellular environment and is activated. Many studies have revealed cross-talk between the JA and SA pathways, and frequently disruptions of one pathway have been reported to lead to up-regulation of the other (15). However, the relationship between the JA and SA pathways is more complicated than simple antagonism, and when applied at lower concentrations, JA and SA have been reported to work synergistically, inducing expression of both *PDF1.2* and *PR-1* (16).

***At*Pep-Induced *PR-1* and *PDF1.2* Expression Requires Hydrogen Peroxide Production.** Hydrogen peroxide production is a component of many characterized defense signaling processes (17-20). Supplying diphenylene iodonium chloride, an inhibitor of the NADPH oxidase-generated hydrogen peroxide precursor superoxide (21), to excised leaves, together with *At*Pep1, totally blocks *PDF1.2* expression (7). Supplying diphenylene iodonium chloride to *Arabidopsis* leaves, along with each of the other five *At*Pep peptides, blocked expression of *PR-1* and *PDF1.2* by each peptide, compared with wild-type plants (data not shown). These results indicated that hydrogen peroxide production was essential to *At*Pep-mediated expression of both the JA/Et and SA defense signaling pathways. Hydrogen peroxide is produced in the apoplast and its role in the innate immune response is not clear (18). The inhibition of hydrogen peroxide production and defense gene activation appears to be related to the early active oxygen burst, and hydrogen peroxide may behave as a "second messenger" in regulating defense-gene transcription in mesophyll cells, as found in tomato leaves (19, 22). The role of hydrogen peroxide in the signaling pathways induced by *At*Pep peptides should be investigated further.

Table 1. Transgenic plants constitutively expressing *PROPEP* peptide precursor genes in both *PR-1* and *PDF1.2*

	Relative expression levels			
Transgenic plants	*PROPEP1*	*PROPEP2*	*PR-1*	*PDF1.2*
35S::PROPEP1	17.2 ± 1.7		3.2 ± 1.9	4.4 ± 0.5
35S::PROPEP2		76.7 ± 23.8	3.4 ± 2.0	4.3 ± 1.3

Relative expression of *PR-1* and *PDF1.2* genes in *35S::PROPEP* plants as compared with basal levels in untreated wild-type plants is shown. Comparative expression levels were determined by semiquantitative RT-PCR analysis with β-tubulin expression as a control. Results are derived from four different experiments using at least six independent lines per construct.

Overexpression of *PROPEP* Genes in Transgenic *Arabidopsis* Plants Produces a Constitutive Expression of both *PR-1* and *PDF1.2* in Leaves. Transgenic lines of *Arabidopsis* expressing *35S::PROPEP1* and *35S::PROPEP2* were shown to have increased resistance toward an oomycete, *Pythium irregulare* (7). Here, we report that six independent transgenic lines expressing *35S::PROPEP1* or *35S::PROPEP2* express *PDF1.2* and *PR-1* in leaves at levels higher than those found in wild-type plants (Table 1). These results correlated with the data in Fig. 2 *A* and *B*, in which *At*Pep1 and *At*Pep2 induce expression of both *PDF1.2* and *PR-1*.

In Fig. 5 is a proposed model that includes the data presented here with recent data for the innate immune responses of *Arabidopsis* in response to the bacterial PAMPs flg22 and elf18, in which the peptides interact with receptors in *Arabidopsis* to activate defense genes that regulate both the JA/Et and SA pathways (3, 6, 23, 24), inducing the expression of *PDF1.2* and *PR* protein genes, respectively. How these two pathways are coordinated in response to individual PAMPs has not been explained. However, flg22 and elf18 strongly activate the expression of *PROPEP2*, *PROPEP3*, and their receptor, *PEPR1* (At1g73080) (3, 24). The expression of these genes would result in the production of *At*Pep peptides that can amplify signaling for both the JA/Et and SA pathways and expression of defense genes of each pathway. This could explain how PAMPs such as flg22 and elf18 can amplify multiple defense signaling pathways. *PROPEP* orthologs have been identified in numerous plant species of diverse families, suggesting that a similar amplification mechanism may be found throughout the plant kingdom.

In summary, we present evidence to support the role of peptides derived from *PROPEP* genes as endogenous elicitors that are generated in response to pathogens and their PAMPs. The peptides have the property of inducing expression of their own genes to initiate a feedback mechanism to amplify the original PAMP signals, thus being PAMP amplifiers. A simplified model is presented in Fig. 5 that incorporates the data reported here and elsewhere (25, 26) into known components of the innate immune response. A similar feedback amplification mechanism is also found in wound signaling in tomato plants, where *prosystemin* (27) and *LepreproHypSys* (28) are expressed in response to wounding to generate peptides that amplify the octadecanoid signaling pathway enzymes and therefore JA in vascular bundle cells (29-32). A recent report has suggested that the systemic signal in *Arabidopsis* that activates the systemic acquired resistance (SAR) pathway may be jasmonic acid or a derivative (33). It is possible that *At*Pep peptides, as amplifiers of both JA/Et and SA pathways, may play a role in systemic signaling in *Arabidopsis* and throughout the plant kingdom in species where *PROPEP* orthologs are found, to both amplify defense signaling by maintaining levels of JA and SA for long-distance signaling.

Materials and Methods

Plant Propagation. Seeds of the *fad3-2 fad7-2 fad8* triple-mutant plant were provided by J. Browse (Washington State University). *ein2-1* and *npr1-1* mutant seeds were obtained from *Arabidopsis* Biological Resource Center (Ohio State University, Columbus,

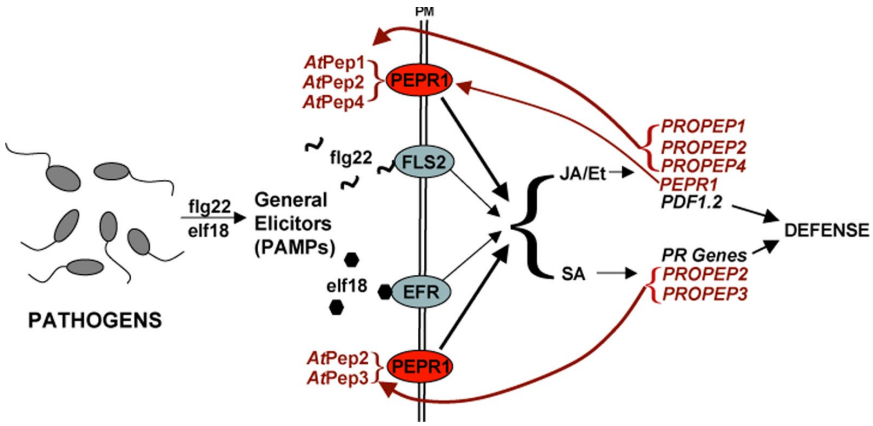

Fig. 5. A model for the amplification of signaling pathways for PAMPs and *At*Pep peptides in *Arabidopsis*. The PAMPs flg22 and elf18 are perceived by their respective receptors, FLS2 and EFR (25, 26), to initiate signaling through the JA/Et and SA pathways to express the defense protein genes *PDF1.2*, *PR-1*, and *PROPEP*. Peptides derived from the *PROPEP* genes are transported to the apoplast, where they can interact with the cell-surface receptor PEPR1 to further amplify signaling.

OH) and *sid2-2* seeds were provided by F. Ausubel (Massachusetts General Hospital, Boston, MA). All mutant seed and wild-type *Arabidopsis thaliana* (ecotype Columbia) seeds were stored at 4°C until planting.

Wild-type, *ein2-1*, *fad* triple-mutant, and *npr1-1* plants were planted in twice-autoclaved potting soil with four seeds per 8 cm^3 pot. The pots were covered with cheesecloth to retain moisture, and the seeds germinated for 6 days under low light at ≈18°C. The seedlings were moved to growth chambers where they were grown under a 16-h day-length of 250 microeinstein/m^2·s of light (1 einstein = 1 mol of photons) at 21°C and watered from the bottom of the pot daily. The *sid2-2* seed was planted into twice-autoclaved Metro Mix 360 (Sun Gro Horticulture Distribution, Bellevue, WA) in 4-inch pots. The mix in each pot was covered by a piece of cheesecloth and topped with a thin layer of soil. Four *sid2-2* seeds per pot were distributed evenly on the soil and grown under the same conditions as above.

Plant Hormone Treatments. Plants were sprayed with solutions of either 625 μM MeJA (Bedoukian Research, Danbury, CT) or 2 mM MeSA (Sigma–Aldrich, St. Louis, MO). Both solutions were prepared in 0.1% Triton X-100 (Sigma–Aldrich). The treated plants and control plants were incubated in closed Plexiglas boxes in separate growth chambers.

Excised-Leaf Assays. *At*Pep peptides were assayed for ability to induce defense-related gene expression in excised *Arabidopsis* leaves as described (7). Briefly, leaves from 3- to 4-week-old plants were excised at the petiole and placed in 800-μl centrifuge tubes containing either 20 nM solutions of each peptide, or for control leaves, distilled water. To block H_2O_2-dependent gene expression in leaves, 100 μM diphenylene iodonium chloride (Sigma–Aldrich), an inhibitor of H_2O_2 production (19, 21), was supplied to excised leaves through the cut petiole in small vials with and without *At*Pep peptides and incubated in Plexiglas boxes in a growth chamber for 2 h under constant light of 200 microeinstein/m^2·s at 21°C. To terminate the assays, leaves were frozen in liquid nitrogen.

Semiquantitative RT-PCR Analysis of Relative Gene Expression Levels. Semiquantitative RT-PCR was performed as described (1). RNA was isolated by using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA), and 2 μg of RNA template per reaction was reverse transcribed with the RETROscript kit (Ambion, Austin, TX). For amplification, 2.5 μl of the cDNA generated by the reverse transcription reactions was used as template for 25 μl of PCRs that used Ex Taq Hot Start polymerase and reagents (Fisher Scientific, Pittsburgh, PA).

All primers designed to amplify intron-spanning fragments of the six *PROPEP* transcripts were used at a concentration of 1 μM. These primers and primers to amplify fragments of the *PDF1.2* transcripts (At5g44420), *PR-1* transcripts (At2g14610), and β-tubulin transcripts (At5g62690) are as follows: *PROPEP*1 forward primer, 5′-CTT ATC AGA TCT CAA TGG AGA AAT C-3′, and reverse primer, 5′-CAA TGT AAC TTA AAG TGC CTA ATT ATG-3′; *PROPEP*2 forward primer, 5′-TCA CCA AAC TAT TGG ATT TCA A-3′, and reverse primer, 5′-GAC TCA ATT TTT GAC TTC TTA ATC C-3′; *PROPEP*3 forward primer, 5′-CAA CGA TGG AGA ATC TCA GA-3′, and reverse primer, 5′-CTA ATT GTG TTT GCC TCC TTT-3′; *PROPEP*4 forward primer, 5′-AAC TTA GCT CTC ACG AAG CA-3′, and reverse primer, 5′-AAA AAT AAA GGA CTC GTA GGA GTT-3′; *PROPEP*5 forward primer, 5′-GAA GAT GCA GCA AGA GAG AG-3′, and reverse primer, 5′-TAG TTA CAT GTC GTA GTC GTT AAC TC-3′; *PROPEP*6 forward primer, 5′-ATG GAA GTT AAT GGA GAA GAA GA-3′, and reverse primer, 5′-ATT GTT TTG ACC AGG TCG T-3′; *PDF1.2* forward primer, 5′-ATG GCT AAG TTT GCT TCC A-3′, and reverse primer, 5′-TTA ACA TGG GAC GTA ACA GAT AC-3′; *PR-1* forward primer, 5′-GGA GCT ACG CAG AAC AAC TA-3′, and reverse primer, 5′-AGT ATG GCT TCT CGT TCA CA-3′; and β-tubulin forward primer, 5′-CAA CGC TAC TCT GTC TGT CC-3′, and reverse primer, 5′-TCT GTG AAT TCC ATC TCG TC-3′.

Reactions to analyze *PROPEP* or *PDF1.2* transcript abundance were performed with an initial denaturing/polymerase activating step of 5 min at 94°C followed by 31 repetitions of the following three steps: a 30-s denaturation phase at 94°C, a 30-s annealing period at 55.5°C, and a 1-min elongation step at 72°C. The amplification program was terminated with a 10-min final 72°C elongation phase. *PR-1* and β-tubulin reactions were amplified with a shorter program of 29 rather than 31 cycles.

Products of each reaction were separated by electrophoresis and visualized on a Bio Imaging System by using GeneSnap version 6.00.26 software (Syngene, Frederick, MD). Gel images were analyzed with GeneTools analysis software version 3.02.00 (Syngene). The relative intensity of each band was calculated by normalization to a tubulin band to yield a numerical ratio. The semiquantitative PCR method was per-

formed in duplicate for every sample, and the original RNA extraction experiments were performed in triplicate to calculate average ratios.

Construction of Vectors and Plant Transformation. Cassettes containing *PROPEP* genes under the control of the cauliflower mosaic virus 35S promoter were generated with the pART-7/pBART binary vector system (34), as described (7), and used to transform wild-type *Arabidopsis* (ecotype Columbia) plants (35).

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1. Janeway CA, Jr, Medzhitov R (2002) *Annu Rev Immunol* 20:197-216.
2. Brunner F, Rosahl S, Lee J, Rudd JJ, Geiler C, Kauppinen S, Rasmussen G, Scheel D, Nürnberger T (2002) *EMBO J* 21:6681-6688.
3. Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JDG, Felix G, Boller T (2004) *Nature* 428:764-767.
4. Nürnberger T, Lipka V (2005) *Mol Plant Pathol* 6:335-345.
5. Ausubel FM (2005) *Nature Immunol* 6:973-979.
6. Zipfel C, Felix G (2005) *Curr Opin Plant Biol* 8:353-360.
7. Huffaker A, Pearce G, Ryan CA (2006) *Proc Natl Acad Sci USA* 103:10098-10103.
8. Yamaguchi Y, Pearce G, Ryan CA (2006) *Proc Natl Acad Sci USA* 103:10104-10109.
9. Toufighi K, Brady SM, Austin R, Ly E, Provart NJ (2005) *Plant J* 43:153-163.
10. Craigon DJ, James N, Okyere J, Higgins J, Jotham J, May S (2004) *Nucleic Acids Res* 32:D575–D577.
11. Guzman P, Ecker JR (1990) *Plant Cell* 2:513-523.
12. McConn M, Browse J (1996) *Plant Cell* 8:403-416.
13. Cao H, Bowling SA, Gordon AS, Dong X (1994) *Plant Cell* 6:1583-1592.
14. Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) *Nature* 414:562-565.
15. Kunkel BN, Brooks DM (2002) *Curr Opin Plant Biol* 5:325-331.
16. Mur LA, Kenton P, Atzorn R, Miersch O, Wasternack C (2006) *Plant Physiol* 140:249-262.
17. Laloi C, Apel K, Danon A (2004) *Curr Opin Plant Biol* 7:323-328.
18. Torres MA, Jones JD, Dangl JL (2006) *Plant Physiol* 141:373-378.
18. Orozco-Cárdenas ML, Narváez-Vásquez J, Ryan CA (2001) *Plant Cell* 13:179-191.
20. Coego A, Ramirez V, Gil MJ, Flors V, Mauch-Mani B, Vera P (2005) *Plant Cell* 17:2123-2137.
21. O'Donnell VB, Tew DG, Jones OTG, England PJ (1993) *Biochem J* 290:41-49.
22. Browse J (2005) *Vitam Horm* (*San Francisco*) 72:431-456.
23. Gómez-Gómez L, Felix G, Boller T (1999) *Plant J* 18:277-284.
24. Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JDG, Boller T, Felix G (2006) *Cell* 125:749-760.
25. Gómez-Gómez L, Boller T (2002) *Trends Plant Sci* 7:251-257.
26. Kunze G, Zipfel C, Silke R, Niehaus K, Boller T, Felix G (2004) *Plant Cell* 16:3496-3507.
27. McGurl B, Pearce G, Orozco-Cárdenas ML, Ryan CA (1992) *Science* 255:1570-1573.
28. Pearce G, Ryan CA (2003) *J Biol Chem* 278:30044-30050.
29. Narváez-Vásquez J, Ryan CA (2004) *Planta* 218:360-369.
30. Narváez-Vásquez J, Pearce G, Ryan CA (2005) *Proc Natl Acad Sci USA* 102:12974-12977.
31. Wasternack C, Stenzel I, Hause B, Hause G, Kutter C, Maucher H, Neumerkel J, Feussner I, Miersch O (2006) *J Plant Physiol* 163:297-306.
32. Schilmiller AL, Howe G (2005) *Curr Opin Plant Biol* 8:369-377.
33. Truman W, Bennett MH, Kubigsteltig I, Turnbull C, Grant M (2007) *Proc Natl Acad Sci USA* 104:1075-1080.
34. Gleave AP (1992) *Plant Mol Biol* 20:1203-1207.
35. Clough SJ, Bent AF (1998) *Plant J* 16:735-743.