

# The Transcriptional Innate Immune Response to flg22. Interplay and Overlap with Avr Gene-Dependent Defense Responses and Bacterial Pathogenesis<sup>1[w]</sup>

Lionel Navarro<sup>2</sup>, Cyril Zipfel<sup>2</sup>, Owen Rowland<sup>3</sup>, Ingo Keller, Silke Robatzek, Thomas Boller, and Jonathan D.G. Jones\*

The Sainsbury Laboratory, John Innes Centre, Norwich Research Park, United Kingdom (L.N., O.R., I.K., J.D.G.J.); and Friedrich Miescher-Institut for Biomedical Research, CH-4058 Basel, Switzerland (C.Z., S.R., T.B.)

Animals and plants carry recognition systems to sense bacterial flagellin. Flagellin perception in Arabidopsis involves FLS2, a Leu-rich-repeat receptor kinase. We surveyed the early transcriptional response of Arabidopsis cell cultures and seedlings within 60 min of treatment with flg22, a peptide corresponding to the most conserved domain of flagellin. Using Affymetrix microarrays, approximately 3.0% of 8,200 genes displayed transcript level changes in flg22 elicited suspension cultures and seedlings. *FLARE* (Flagellin Rapidly Elicited) genes mostly encode signaling components, such as transcription factors, protein kinases/phosphatases, and proteins that regulate protein turnover. Approximately 80% of flg22-induced genes were also up-regulated in Arabidopsis seedlings treated with cycloheximide. This suggests that many *FLARE* genes are negatively regulated by rapidly turned-over repressor proteins. Twenty-one tobacco Avr9/Cf-9 rapidly elicited (*ACRE*) cDNA full-length sequences were used to search for their Arabidopsis orthologs (*AtACRE*). We identified either single or multiple putative orthologs for 17 *ACRE* genes. For 13 of these *ACRE* genes, at least one Arabidopsis ortholog was induced in flg22-elicited Arabidopsis suspension cells and seedlings. This result revealed a substantial overlap between the Arabidopsis flg22 response and the tobacco Avr9 race-specific defense response. We also compared *FLARE* gene sets and genes induced in basal or gene-for-gene interactions upon different *Pseudomonas syringae* treatments, and infer that *Pseudomonas syringae* pv *tomato* represses the flagellin-initiated defense response.

Plants and animals mount defense responses upon recognition of numerous pathogen-derived molecules. These pathogen-associated molecular patterns (PAMPs) include bacterial cell wall components such as lipopolysaccharide (Ulevitch and Tobias, 1999). PAMPs are (1) highly conserved (2) present in different organisms and (3) usually play a pivotal role for the life of the microorganism (Janeway and Medzhitov, 1998). In mammals, the perception of PAMPs occurs through Toll-like receptors (TLRs). For instance, in mice, the innate immune response is activated through perception of the Salmonella flagellin by the TLR5 receptor (Hayashi et al., 2001). Several plant species, including Arabidopsis, have a specific recognition system for a conserved, 22-amino acid motif (flg22) of the bacterial flagellin (Felix et al., 1999). The Arabi-

dopsis innate immune response to flg22 involves a host recognition protein complex that contains the FLS2 Leu rich repeat (LRR) receptor kinase (Gómez-Gómez et al., 2001). The flg22-FLS2 interaction leads to production of reactive oxygen species (ROS), medium alkalization, activation of mitogen-activated protein (MAP) kinases, and induction of pathogen-responsive genes (Felix et al., 1999; Gómez-Gómez et al., 1999; Nühse et al., 2000; Asai et al., 2002).

In gene-for-gene relationships, plants carrying a resistance (*R*) gene resist pathogen races with the corresponding avirulence (*Avr*) gene (Flor, 1971; Keen, 1990). This specific recognition leads to activation of defense responses and local cell death referred to as the hypersensitive response (HR). A well-characterized example of HR elicitation through gene-for-gene interaction is provided by the tomato (*Lycopersicon esculentum*) Cf-9 gene, which confers resistance to races of the fungus *Cladosporium fulvum* expressing the Avr9 gene (Van den Ackerveken et al., 1992). The product of Avr9 is secreted and subsequently processed by fungal and plant proteases to produce a peptide of 28 amino acids (Joosten et al., 1994). Treatment of leaves of Cf9 tomato or transgenic Cf9 tobacco (*Nicotiana tabacum*) with the Avr9 peptide induces HR within 24 h (Hammond-Kosack et al., 1998). In addition, Avr9-treated Cf9 tobacco cell cultures show rapid production of ROS and activation of MAP kinases and calcium-dependent protein kinases (CDPKs;

<sup>1</sup> This work was supported by the Gatsby Charitable Foundation (to L.N. and O.R.), by a fellowship from the Human Frontiers Science Program (to O.R.), by the Novartis Research Foundation (to C.Z. and S.R.), and by a grant of the Swiss National Foundation (to T.B.).

<sup>2</sup> These authors contributed equally to the paper.

<sup>3</sup> Present address: Department of Botany, University of British Columbia, Vancouver, Canada.

\* Corresponding author; e-mail jonathan.jones@sainsbury-laboratory.ac.uk; fax 44-1603-450-011.

<sup>[w]</sup>The online version of this article contains Web-only data.

Article, publication date, and citation information can be found at [www.plantphysiol.org/cgi/doi/10.1104/pp.103.036749](http://www.plantphysiol.org/cgi/doi/10.1104/pp.103.036749).

Romeis et al., 1999, 2000). Gene expression profiling of Avr9-treated Cf9 tobacco cells revealed a set of Avr9/Cf-9 rapidly elicited (*ACRE*) genes induced within 15 to 30 min after elicitation (Durrant et al., 2000).

Bacterial plant pathogens can also be recognized in a gene-for-gene manner. Bacterial Avr proteins are translocated into the host cells through a type III protein secretion system (Galan and Collmer, 1999) which, in the case of *Pseudomonas syringae* DC3000, is thought to deliver more than 30 effector proteins (Boch et al., 2002; Collmer et al., 2002; Fouts et al., 2002; Guttman et al., 2002; Petnicki-Ocwieja et al., 2002; Zwiesler-Vollick et al., 2002). AvrRPM1 and AvrRpt2 from *P. syringae* provide examples of such type III effector proteins that are recognized by the products of the *RPM1* and *RPS2* resistance genes, respectively (Dangl et al., 1992; Innes et al., 1993). This recognition initiates the plant HR response through modification or loss of the host RIN4 protein (Mackey et al., 2002; Mackey et al., 2003; Axtell and Staskawicz, 2003). Although the mechanisms of bacterial Avr defense activation is becoming clearer, very little is known about the potential connection between race-specific and PAMP-mediated innate immune responses to bacterial pathogens.

Most plants are resistant to most pathogens through a basal defense mechanism referred to as nonhost resistance, which is based on both constitutive and inducible defense responses. For instance, the nonhost bacterium *P. syringae* pv *tabaci* induces accumulation of defense transcripts in *Phaseolus vulgaris*, leading to antimicrobial phytoalexin production (Jakobek et al., 1993). Interestingly, type III secretion system mutants of the same bacterial strain trigger the same set of genes in *Phaseolus vulgaris* (Jakobek et al., 1993), suggesting that general elicitors such as PAMPs (e.g. flg22) are likely to play a crucial, albeit yet uncharacterized, role in elicitation of nonhost resistance.

The goal of this study was to investigate the possible connections between innate immunity, race-specific, and nonhost types of resistance responses. Using a high-density oligonucleotide microarray (Affymetrix, La Jolla, CA), we studied the rapid changes in gene expression that occur in Arabidopsis cell cultures and seedlings treated with the flg22 peptide. We found that these flagellin rapidly elicited (*FLARE*) genes mostly encode signaling components. The flg22-rapidly elicited genes in cell cultures were called *cFLARE* genes and in seedlings *sFLARE* genes. The majority of these genes were also up-regulated upon treatments with the protein synthesis inhibitor cycloheximide (CHX), suggesting that *FLARE* genes are negatively regulated by rapidly turned-over repressor proteins. Analysis of a set of Arabidopsis *ACRE* orthologs revealed a substantial overlap between the Avr9 race-specific response in tobacco and the flg22-elicited innate immune response in Arabidopsis, suggesting that at least some polymorphic race-specific resistance mechanisms have evolved from mechanisms that recognize PAMPs. Finally, a comparison of genes that were up-

regulated upon treatments with either virulent, avirulent, or nonhost *P. syringae* strains revealed that (1) genes induced in nonhost interactions might be regulated through PAMP perception, (2) some type III effector proteins could suppress PAMP-induced genes, and (3) Avr proteins, if recognized through an *R* gene, might positively regulate the PAMP-mediated innate immune response.

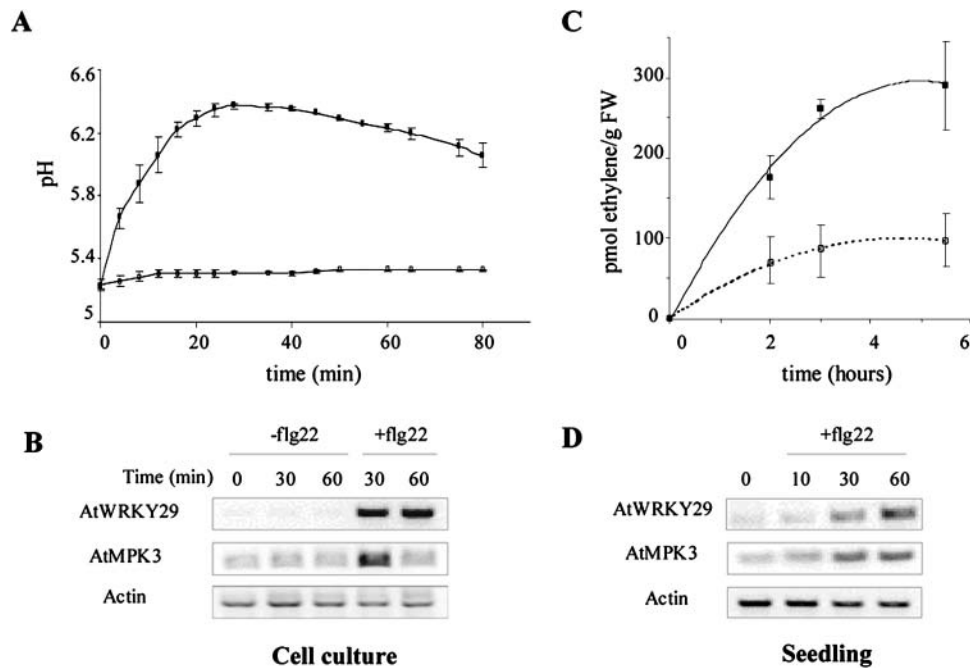
## RESULTS

### Validation of Cell Culture and Seedling Systems for flg22 Inducibility

To monitor gene expression changes in response to flg22, cell suspension cultures of Arabidopsis ecotype Landsberg *erecta* (*Ler*) were exposed in two independent experiments to 100 nM flg22. RNA was prepared from cells 30 and 60 min after elicitation. Control samples were taken from cultures treated with dimethyl sulfoxide, and from untreated cell cultures. Elicitors, such as flg22, induce medium alkalinization and ethylene production (Felix et al., 1999; Gómez-Gómez et al., 1999). The pH in the extracellular medium of the cell cultures was monitored upon flg22 addition and a very reproducible response was observed (Fig. 1A; Felix et al., 1999). In parallel, two independent sets of 2-week-old Arabidopsis ecotype Columbia (Col-0) seedlings were incubated with 10  $\mu$ M flg22 for 30 min, and total RNA extracted. To confirm elicitation, the flg22-induced production of ethylene was measured (Fig. 1C). Moreover, reverse transcription (RT)-PCR of selected genes such as *AtWRKY29* (At4g23550), previously described to be rapidly flg22 inducible in Arabidopsis protoplasts (Asai et al., 2002), and *AtMPK3* (At3g45640), the Arabidopsis ortholog of *WIPK* (Romeis et al., 2000) that is rapidly induced in Cf-9-tobacco suspension cells upon Avr9 treatment, showed the flg22-inducibility of both systems (Fig. 1, B and D).

### Identification and Classification of Early flg22-Regulated Genes

We used high-density oligonucleotide arrays (Affymetrix) to study early flg22-induced changes in gene expression and to identify flg22-rapidly elicited (*FLARE*) genes. The arrays contain probe sets for about 8,200 different Arabidopsis genes (Zhu and Wang, 2000). Biotin-labeled cRNA representing each time point was hybridized individually. To identify the induced or repressed genes in duplicate experiments, we used quantitative and qualitative criteria that were applied individually to the data set at each time point of the time course. Genes were considered as up- or down-regulated if their expression level deviated (positively or negatively) more than 2.5-fold upon elicitor treatment, and designated I for increase and D for decrease based on Wilcoxon's signed-rank test



**Figure 1.** Responsiveness of Arabidopsis cell cultures and seedlings to flg22 elicitor. A, Extracellular medium alkalization in Arabidopsis cell culture. The pH of the cell culture extracellular medium was measured with glass electrode. White boxes represent control cell cultures and black boxes represent flg22-treated cell cultures. Error bars correspond to *SD* observed in two independent experiments that were used for the microarray analysis. B, RT-PCR of *AtWRKY29* (At4g23550) and *AtMPK3* (At3g45640) in Arabidopsis cell culture. RT-PCR of a constitutively expressed actin gene (At5g09810) was also performed to control equal cDNA amount in each reaction (bottom lane). C, Ethylene production in Arabidopsis seedlings. Increase of ethylene was measured by gas chromatography. White boxes represent control seedlings and black boxes represent flg22-treated seedlings. Error bars correspond to *SD*. D, RT-PCR of *AtWRKY29* (At4g23550) and *AtMPK3* (At3g45640) in Arabidopsis seedlings. RT-PCR of a constitutively expressed actin gene (At5g09810) was performed to control equal cDNA amount in each reaction (bottom lane).

performed using Affymetrix software (see “Materials and Methods” for details and Liu et al., 2002).

In our *Ler* cell culture assay, 225 *cFLARE* distinct genes (approximately 2.8%) showed significant changes in mRNA level over 60 min (see Supplemental Table I, which can be viewed at [www.plantphysiol.org](http://www.plantphysiol.org)). Ninety-three genes were significantly induced, whereas only six genes were repressed at both time-points (see Supplemental Tables II and III). Analysis of our seedling data revealed 252 *sFLARE* distinct genes that were significantly altered upon flg22 elicitation (see Supplemental Table IV).

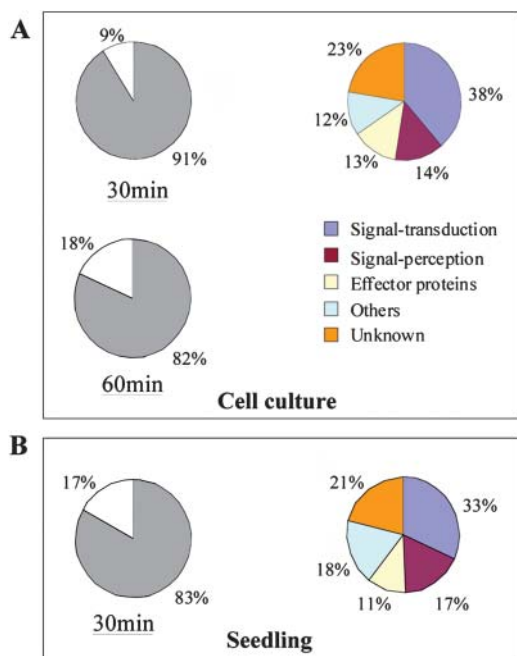
Overall, 80% of the *FLARE* genes are currently annotated as encoding proteins of known or predicted function. We functionally classified these as signal transduction-related, signal-perception-related, effector proteins, and others (see Supplemental Tables V–VIII and Fig. 2, A and B). Among the signal transduction-related genes, many are transcription factors, which represent 43% and 52% of the overall signaling class in suspension cells and seedlings, respectively, and include several WRKY transcription factors (Table I). Among those, we identified *AtWRKY6* (At1g62300; Robatzek and Somssich, 2002) as well as *AtWRKY22* and *AtWRKY29* (At4g01250 and At4g23550), whose

overexpression increased resistance to both bacterial and fungal pathogens (Asai et al., 2002). In addition, six additional WRKY transcription factors were newly identified as flg22-induced genes and are likely to be involved in plant defense.

A number of *FLARE* genes encode proteins involved in regulating protein turnover such as U-box and RING zinc-finger proteins (Table I). This is consistent with other results indicating an important role for protein turnover in derepressing plant defenses (Peart et al., 2002). Intriguingly, many auxin signaling-related genes were down-regulated during the flg22 response (Table I).

The group of signal-perception-related genes includes resistance-like genes and genes required for resistance (Table II). Among those, we identified *RPS2* that confers resistance to *P. syringae* carrying *AvrRpt2* (Kunkel et al., 1993). Strikingly, this class of *FLARE* genes also includes a large number of receptor like-kinases (RLKs) with various extracellular domains.

The full complement of *FLARE* genes also comprises some which might be directly involved in halting the growth of pathogens (effector class), e.g. enzymes involved in phenylpropanoid metabolism (see Supplemental Table VII).



**Figure 2.** Abundance of flg22-regulated genes. Percentage distribution of Arabidopsis cell culture (A) and seedlings (B), flg22-activated (gray) and repressed (white) genes, and their classification in functional categories.

### Differential Expression of *FLARE* Genes between Cell Cultures and Seedlings

We found approximately 70% of the *cFLARE* genes in 30-min treated cell cultures were also significantly induced in flg22 treated seedlings (see Supplemental Tables I and IV). In contrast, we observed that approximately 40% of the *sFLARE* genes identified in elicited seedlings were also up-regulated in the 30-min treated cell cultures highlighting a larger set of flg22 regulated genes in the seedling system (see Supplemental Table IX). Only one gene, encoding a putative calcium-dependent protein kinase (*At1g08650*), was down-regulated upon flg22 treatment in both Arabidopsis suspension cells and seedlings (see Supplemental Table X). Most auxin signaling-related genes revealed a similar repression profile in both systems, but none of these repressed genes were identical (Table I). These observations might not only be due to different flg22 concentrations used, but may also result from either the use of different ecotypes or different experimental systems. To address this, we performed RT-PCR on *PAL2* (*At3g53260*), *AtMYB2* (*At2g47190*), and *4CL* (*At1g51680*) on Col-0 cell cultures and *Ler* cell cultures elicited with 100 nM of flg22 peptide over a 1-h time course. These genes were chosen based on their high inducibility in treated *Ler* suspension cells and no transcript change in treated Col-0 seedlings. Our results showed a similar pattern of induction in both Col-0 and *Ler* cell cultures (Fig. 3). In addition, no transcript alteration of these genes was detected in *Ler* seedlings treated with 10  $\mu$ M flg22 peptide (data not

shown). These data suggest that the differences in gene expression between *Ler* suspension cells versus Col-0 seedlings are mostly due to differences between cell cultures and seedlings rather than to differences between ecotypes.

### Comparison of *ACRE* and *FLARE* Gene Complements

Both *FLARE* genes and *ACRE* (Durrant et al., 2000) genes comprise approximately 1% of expressed genes after 30-min treatment with flg22 in Arabidopsis and Avr9 in tobacco cell cultures. Moreover, in both systems we observed that more transcripts are induced than repressed (data not shown). To more precisely compare the rapid transcript alterations, we concentrated on flg22-induced expression changes of probable Arabidopsis orthologs of *ACRE* genes (*AtACRE* genes). Twenty full-length *ACRE* cDNA sequences were used to search for Arabidopsis orthologs, of which 10 *ACRE* genes were derived from cDNA library screening (Durrant et al., 2000) and the remainder from 3' and 5' RACE amplification (O. Rowland, A.A. Ludwig, C. Merrick, F. Baillieul, F. Tracy, W. Durrant, H. Yoshioka, and J.D.G. Jones, unpublished data). We also included *NtCDPK2* that was induced 15 min after elicitation of Cf9-tobacco cell cultures with Avr9 peptide (Romeis et al., 2000). Whereas in some cases single putative Arabidopsis orthologs could be identified, such as *AtACRE276*, other tobacco *ACRE* cDNA sequences revealed homologies to several Arabidopsis counterparts (Table III). For example, the tobacco *ACRE189* full-length cDNA displayed a high sequence similarity to 4 putative Arabidopsis F-box genes, any of which could represent the functional Arabidopsis ortholog. The identities of the *AtACRE* candidates were confirmed using the TBLASTN program from The Institute for Genomic Research (TIGR) orthologous gene alignment database (<http://www.tigr.org/tdb/toga/toga.shtml>). Seventeen out of 21 tobacco full-length cDNAs showed high homology with either a single or several Arabidopsis counterparts. In total, these genes represent 32 putative *AtACRE* candidates. Since one-third of the Arabidopsis genome is covered in the Affymetrix GeneChip Arabidopsis genome array, only 14 out of the 32 *AtACRE* genes were present on the array, and their expression patterns were further studied. The remaining *AtACRE* candidates were profiled using semi-quantitative RT-PCR.

With the exceptions of tobacco *ACRE137*, *ACRE141*, *ACRE216*, and *ACRE275*, at least one of the Arabidopsis *ACRE* orthologs was induced in flg22-elicited Arabidopsis suspension cells (Fig. 4A). The overall expression analysis revealed 13 rapidly and transiently flg22-induced genes and 5 progressively induced genes (Table III; Fig. 4A). Whereas *CPK1* (*At5g04870*) was not induced based on our microarray analysis filters, we observed a slight induction of this gene in elicited cell cultures (Fig. 4A). In elicited seedlings, most of the *AtACRE* genes displayed a very

**Table 1.** Highlights of *FLARE* genes with known or putative roles in signal transduction

Average relative values of flg22-treated samples, compared to control samples, from two independent experiments. Numbers show the factor of change between control and treatments; positive values represent up-regulation (e.g. 5 = 5-fold increase), negative values down-regulation (e.g. -5 = 5-fold decrease). Expression changes of less than 2-fold between control and treatment are indicated by a dash (-).

Gene Description	AGI Number	Change after Treatment		
		Cells	Cells	Seedlings
		30 min	60 min	30 min
<b>WRKY transcription factors</b>				
AtWRKY29	At4g23550	6.1	44	4.7
AtWRKY53	At4g23810	22	9.8	34.6
AtWRKY28	At4g18170	-	-	32.2
AtWRKY22	At4g01250	24.5	14.4	24.1
AtWRKY33	At2g38470	4.6	12.3	28.6
AtWRKY11	At4g31550	5	7	13.0
AtWRKY15	At2g23320	-	2.7	4.3
AtWRKY6	At1g62300	-	2.7	7.3
AtWRKY7	At4g24240	-	-	3.1
<b>Protein turnover</b>				
RING-H2 finger protein, RHA3b	At4g35480	-	-	28.0
RING-H2 finger protein, RHA1b	At4g11360	-	9.6	4.6
AtRMA1 protein	At4g03510	-	8.3	-
AtPUB12	At2g28830	2.0	11.5	4.9
Putative RING finger protein	At2g42360	-	4.1	10.3
Putative RING finger protein	At3g16720	3.6	2.7	8.4
AtPUB5	At4g36550	4.5	2.5	5.1
Putative RING finger protein	At4g26400	-	-	4.9
Putative RING finger protein	At2g35000	2.6	3.2	3.9
Putative RING finger protein	At2g42350	-	2.8	-
Similar to RING Zn finger protein	At2g44410	-	2.7	-
RING-H2 finger protein, ATL6	At3g05200	-	-	4.0
<b>Hormone signaling</b>				
Axi 1-like protein	At2g44500	5.9	-	4.7
Putative auxin-regulated protein	At2g46690	-2.6	-	-
Auxin transport protein, PIN3	At1g70940	-	-3.3	-
Early auxin-induced, IAA13	At2g33310	-2.6	-3.2	-
Early auxin-induced, IAA5	At1g15580	-	-6.6	-
Putative auxin-induced protein	At2g16580	-2.1	-9.3	-
Similar to auxin-regulated gene	At4g34750	-	-	-2.7
SAUR-AC1	At4g38850	-	-	-8.0
Putative auxin-induced protein	At2g21210	-	-	-9.0
Auxin-induced protein-like	At4g38840	-	-	-14.2
Putative auxin-induced protein	At4g38860	-	-	-23.6

similar expression pattern to that in suspension cells (Table III; Fig. 4B). Besides the confirmation of our microarray data, these results revealed a substantial overlap between the Avr9 race-specific defense response in tobacco and the flg22-elicited innate immune response in Arabidopsis.

#### Clustering Analysis of *FLARE* Genes in Arabidopsis Suspension Cells

We identified 3 significant clusters of (1) progressively induced genes (110 genes), (2) transiently induced genes (44 genes), and (3) progressively repressed genes (31 genes; see Supplemental Tables XI–XIII). These clusters were identified by subjecting the absolute expression values of the overall *FLARE*

genes over the time course to a self-organizing map (SOM) algorithm using  $3 \times 1$  two-dimensional matrix (see "Materials and Methods" for details). Within the cluster of transiently induced genes, we found the Arabidopsis *ACRE* orthologs *AtACRE1a/b* (At5g47230, At4g17490), *AtACRE111* (At4g25470), *AtACRE132* (At3g16720), *AtACRE231b/c* (At1g70090, At1g24170), *AtACRE264a* (At2g05940), and *AtACRE284a/c* (At2g30020, At2g40180; see Supplemental Table XII). To gain more insight into the *FLARE* gene regulation, we inspected promoter sequences of genes that clustered together with the progressively induced *AtACRE31* ortholog (At4g20780). This task was performed using GENESPRING software and resulted in the identification of 48 candidates within the *AtACRE31* regulon (see Supplemental Table XIV). We

**Table II.** *FLARE* genes with known or putative roles in signal perception

Average relative values of flg22-treated samples, compared to control samples, from two independent experiments. Numbers show the factor of change between control and treatments; positive values represent up-regulation (e.g. 5 = 5-fold increase), negative values down-regulation (e.g. -5 = 5-fold decrease). Expression changes of less than 2-fold between control and treatment are indicated by a dash (-).

Gene Description	AGI Number	Change after Treatment		
		Cells 30 min	Cells 60 min	Seedlings 30 min
Homologs of disease resistance genes				
Similar to TMV resistance protein (tobacco)	At1g65400	27.7	38.4	27.6
Putative nematode-resistance protein	At2g40000	7.5	7.5	22.7
RPS2	At4g26090	-	-	18.0
Similar to RPP8	At3g50950	2.5	6.1	7.8
Similar to TMV resistance protein (tobacco)	At4g36140	-	-	4.3
Similar to RFL1 disease resistance protein	At4g33300	-	-	4.1
Resistance protein RPP5-like	At4g19520	-	-	3.5
TIR Toll/interleukin-1 receptor-like protein	At1g72930	-	-	2.5
Putative disease resistance protein	At2g19780	-	-6.1	-
Homologs of genes required for resistance				
Putative Mlo protein	At2g39200	8	34	13.9
Athsr4	At3g50930	10.9	22.2	9.1
Similar to Mlo protein	At1g61560	5.2	14.9	10.9
NDR1	At3g20600	-	-	6.3
Similar to EDS1	At3g52430	-	-	6.2
NDR1/HIN1-like protein	At2g27080	-	-	5.3
Hin1-like protein	At2g35980	2.5	2.8	-
NPR1	At1g64280	-	-	3.0
LSD1	At4g20380	-	-	2.5
Receptor-like kinases				
LRR-RLKs				
Receptor-like kinase (LRR5 <sup>a</sup> )	At2g31880	4.6	7.6	13.4
Receptor-like kinase (LRR22 <sup>a</sup> )	At5g25930	2.7	7.4	11.3
Receptor-like kinase (LRR17 <sup>a</sup> )	At2g02220	2.5	8.7	2.5
Receptor-like kinase (LRR10 <sup>a</sup> )	At4g39270	-	3.8	-
Putative-receptor-like protein kinase (LRR4 <sup>a</sup> )	At2g13790	-	2.7	5.4
Similar to CLV1 receptor kinase (LRR22 <sup>a</sup> )	At1g55610	-	-3.4	-
Receptor-like kinase (LRR6 <sup>a</sup> )	At4g22730	-	-4.7	-
Lectin-RLKs				
Receptor-like kinase (LEC <sup>a</sup> )	At4g02410	-	3.7	7.6
LecRK1 receptor-like kinase (LEC <sup>a</sup> )	At3g59700	2.8	6.9	2.7
Receptor-like kinase (LEC <sup>a</sup> )	At1g70130	7.6	7	-
Receptor-like kinase (LEC <sup>a</sup> )	At4g28350	-	5.5	2.5
Receptor-like kinase (LEC <sup>a</sup> )	At4g29050	-	-	4.4
Lys-RLK				
Receptor-like kinase (Lys <sup>a</sup> )	At2g33580	5.2	3.9	17.7
S-RLKs				
Receptor-like kinase (SD <sup>a</sup> )	At2g19130	-	5.4	17.6
Receptor-like kinase (SD <sup>a</sup> )	At4g32300	2.6	12.8	-
Receptor-like kinase (SD <sup>a</sup> )	At4g21390	-	5.6	-
Receptor-like kinase (SD <sup>a</sup> )	At1g61370	-	-	3.0
DUF26-RLKs				
Receptor-like kinase (DUF26 <sup>a</sup> )	At4g23220	-	-	33.2
Receptor-like kinase (DUF26 <sup>a</sup> ), RLK3	At4g23180	7	20.7	8.3
Receptor-like kinase (DUF26 <sup>a</sup> )	At4g23190	3.2	8.2	9.6
Receptor-like kinase (DUF26 <sup>a</sup> ), RKC1	At4g23280	2.7	10.5	6.5
Receptor-like kinase (DUF26 <sup>a</sup> )	At4g23250	-	5.4	-
Receptor-like kinase (DUF26 <sup>a</sup> )	At4g11890	-	-	7.5
Receptor-like kinase (DUF26 <sup>a</sup> )	At4g23270	-	-	3.9
Receptor-like kinase (DUF26 <sup>a</sup> )	At4g21400	-	-	2.5
K-RLKs				
Receptor-like kinase (K <sup>a</sup> )	At2g17220	2.9	9.1	3.9
Receptor-like kinase (K <sup>a</sup> )	At2g05940	10.7	5.3	3.0

(Table continues on following page.)

**Table II.** (Continued from previous page.)

Gene Description	AGI Number	Change After Treatment		
Receptor-like kinase (RKF3L <sup>a</sup> )	At1g11050	–	–	8.1
Receptor-like kinase (K <sup>a</sup> )	At1g67470	–	–	5.7
Receptor-like kinase (K <sup>a</sup> )	At2g47060	–	2.5	5.0
Receptor-like kinase (K <sup>a</sup> )	At2g39660	–	–	4.1
Receptor-like kinase (K <sup>a</sup> )	At3g09010	–	5.2	2.8
Receptor-like kinase (K <sup>a</sup> )	At2g11520	2.5	–	2.6
Receptor-like kinase (K <sup>a</sup> )	At2g40270	–	–	2.5
Receptor-like kinase (K <sup>a</sup> )	At1g11140	–	–3.2	–
Receptor-like kinase (EXT <sup>a</sup> )	At4g02010	–	–	–2.4

<sup>a</sup>Extracellular domain. The abbreviations for the extracellular domains stand for: LRR, Leu-rich repeat, the numbers refer to the number of repeats; LEC, lectin; SD, S-locus glycoprotein; DUF26 domain of unknown function; K, sequence with no predicted signal motif; EXT, extension.

scanned 1.1-kb ATG-upstream sequences for 5 to 8 bp motifs that are over-represented within the *AtACRE31* regulon using GENESPRING (see “Materials and Methods” for details). As a result, we found a significant increase in the frequency of one of these motifs, namely TTTGAC(T/A), in 28 of the 48 promoters tested (data not shown); the TTTGACT sequence representing the consensus binding site for WRKY transcription factors (Eulgem et al., 2000). In contrast, no over-representation of cis-regulatory elements was detected when we analyzed the promoter sequences of genes that clustered together with the transiently induced *AtACRE1a* ortholog (At5g47230).

To further confirm this statistical analysis we inspected the promoter sequences of the entire set of genes within the *AtACRE31* regulon for over-representation of TTTGACT and TTTGACA sequences as well as other known regulatory elements as previously described (Maleck et al., 2000). Once again, only the W-box and W-box-like element frequencies were at least twice the statistically expected frequency that occurs within a set of 500 promoter sequences from *flg22* non-regulated genes (Table IV). Taken together, our promoter analysis led to the identification of a subset of *FLARE* genes potentially regulated by WRKY transcription factors within the *AtACRE31* regulon and suggests common regulatory processes involved during early race-specific and innate immune responses.

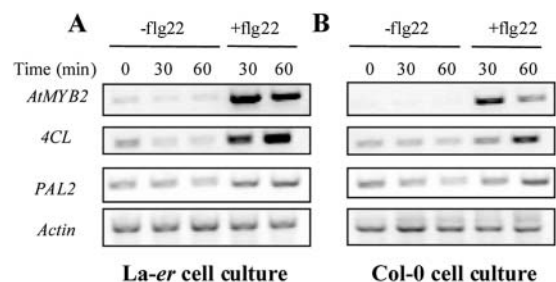
#### Relationship between the *FLARE* Gene Set and Sets of Genes Regulated by *P. syringae* in Nonhost, Compatible, and Incompatible Interactions

To further analyze the relation between *flg22*-triggered early responses and basal or gene-for-gene resistance, we compared the *FLARE* genes to the set of genes regulated by different bacterial treatments in Arabidopsis (Tao et al., 2003). *Pseudomonas* type III effector proteins are delivered into the cytosol of the host cell after a lag of 2 h post inoculation (hpi; Huynh et al., 1989; Grant et al., 2000). Thus, the gene expression dataset from early 3/6 hpi with virulent/avirulent or nonhost *P. syringae* strains (Tao et al.,

2003), is the best available dataset to compare with our *FLARE* gene set regulated within an hour after elicitation.

We decided to focus our comparative analysis on up-regulated genes and carried out a comparison with data sets derived from 3 hpi and 6 hpi of *P. syringae* pv *tomato* (*Pst*), *P. syringae* pv *phaseolicola* (*Psp*), and *P. syringae* pv *tomato* (*Pst*) carrying either *AvrB* or *AvrRpt2* bacterial strains.

As in the Tao et al. (2003) analysis, the ratio of the expression level for each probe set to that in the corresponding water control was calculated at each 3-h and 6-h timepoint. In addition, expression changes derived from plants treated with *Pst* carrying either *AvrB* or *AvrRpt2* genes were divided by expression changes from plants treated with *Pst*. This last selection allows the identification of genes specifically induced by either *AvrB* or *AvrRpt2*. We also selected genes with a minimum fluorescence value of 10 together with a 2.5-fold change ratio (see “Materials and Methods” for details). The overall induced gene sets in nonhost, compatible, and incompatible interactions were then compared to the set of *flg22*-induced genes derived from both elicited cell cultures and seedlings. For this comparative analysis, the same criteria were used to select *flg22*-induced genes.



**Figure 3.** Comparison of *flg22*-regulated candidate genes in Ler and Col-0 cell cultures using semiquantitative RT-PCR. Transcript profiling of *AtMYB2* (At2g47190), *4CL* (At1g51680), and *PAL2* (At3g5326) upon *flg22* elicitation in (A) Ler cell cultures and (B) Col-0 cell cultures. RT-PCR of a constitutively expressed actin gene (At5g09810) was performed to control equal cDNA amount in each reaction (bottom lane).

**Table III.** Identification of putative *Arabidopsis* *ACRE* orthologs and summary of their transcription patterns in response to *flg22*

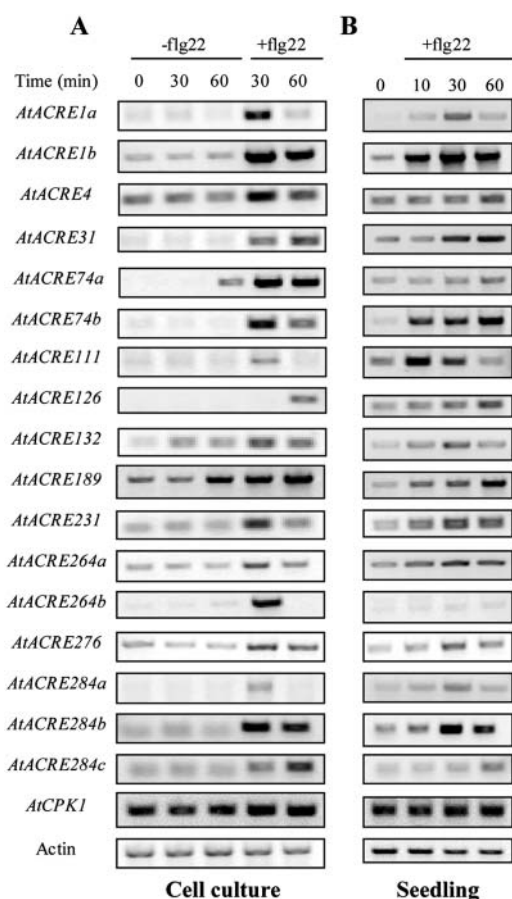
ACRE Number	Genbank Accession Number	Arabidopsis <i>ACRE</i> Orthologs	AGI Number	BLASTX Results	TBLASTN Results (TOGA)	Transcription Patterns <sup>a</sup> Cells/Seedlings
1	AF211527	At-ERF5 ethylene responsive element binding factor	At5g47230	1.5 e <sup>-36</sup>	12.3 e <sup>-36</sup>	TI/TI
		At-ERF6 ethylene responsive element binding factor	At4g17490	4.1 e <sup>-36</sup>	5.4 e <sup>-36</sup>	TI/TI
4	AF211528	Putative disease resistance protein (TIR-NBS-LRR)	At5g17680	4.1 e <sup>-107</sup>	7.1 e <sup>-99</sup>	TI/PI
31	AF211529	Calcium-binding protein-like	At4g20780	1.0 e <sup>-48</sup>	3.2 e <sup>-48</sup>	PI/PI
		Calmodulin-like protein	At5g44460	1.7 e <sup>-46</sup>	4.8 e <sup>-46</sup>	NC/NC
74	AY220484	U-box protein (AtPUB21)	At5g37490	1.0 e <sup>-80</sup>	2.4 e <sup>-47</sup>	TI/PI
		U-box protein (AtPUB20)	At1g66160	3.0 e <sup>-63</sup>	3.8 e <sup>-63</sup>	TI/PI
111	AF211530	DRE binding protein (DREB1A)	At4g25480	3.6 e <sup>-53</sup>	3.8 e <sup>-52</sup>	NC/NC
		DRE binding protein (DREB1B)	At4g25490	1.2 e <sup>-52</sup>	1.4 e <sup>-52</sup>	TI/TI
		DRE binding protein (DREB1C)	At5g51990	5.2 e <sup>-52</sup>	1.8 e <sup>-52</sup>	NC/NC
		DRE binding protein (similar to DREB1C)	At4g25470	8.5 e <sup>-52</sup>	4.2 e <sup>-51</sup>	NC/NC
126	AY220477	AtWRKY72	At5g15130	5.4 e <sup>-41</sup>	7.1 e <sup>-49</sup>	PI/PI
132	AF211532	RING-H2 zinc finger protein ATL3	At1g53820	6.5 e <sup>-34</sup>	4.1 e <sup>-26</sup>	NC/NC
		Putative RING-H2 zinc finger protein	At3g16720	2.9 e <sup>-26</sup>	6.2 e <sup>-26</sup>	TI/TI
137	AF211537	Hypothetical protein	At3g23160	1.5 e <sup>-43</sup>	1.3 e <sup>-43</sup>	NC/NC
141	AY220478	Putative ligand-gated ion channel	At2g29100	3.3 e <sup>-137</sup>	2.1 e <sup>-131</sup>	NC/NC
189	AY220479	F-box protein	At1g47056	3.4 e <sup>-158</sup>	3.9 e <sup>-158</sup>	NC/NC
		SKIP1 interacting partner 2 (SKIP2)	At5g67250	5.6 e <sup>-158</sup>	5.2 e <sup>-158</sup>	PI/PI
		F-box (AtFBL8/AtFBL24)	At4g07400	1.8 e <sup>-152</sup>	4.0 e <sup>-152</sup>	NC/NC
		F-box (AtFBL16)	At3g50080	4.1 e <sup>-146</sup>	4.2 e <sup>-146</sup>	NC/NC
216	AY220480	Putative protein kinase	At2g30260	3.6 e <sup>-131</sup>	3.6 e <sup>-131</sup>	NC/NC
231	AF211536	Glycosyl transferase-like	At3g28340	1.8 e <sup>-127</sup>	2.9 e <sup>-127</sup>	TI/TI
264	AY220481	Putative protein kinase	At2g05940	1.2 e <sup>-155</sup>	1.2 e <sup>-155</sup>	TI/TI
		Ser/Thr protein kinase	At5g35580	2.9 e <sup>-145</sup>	1.1 e <sup>-143</sup>	TI/NC
275	AY220482	Disease resistance protein (Cf-like)	At1g45616	5.0 e <sup>-46</sup>	2.1 e <sup>-46</sup>	NC/NC
276	AY220483	U-box protein (AtPUB17)	At1g29340	1.1 e <sup>-227</sup>	9.7 e <sup>-228</sup>	TI/TI
284	AY220484	Protein phosphatase 2C (PP2C)	At2g30020	4.7 e <sup>-104</sup>	6.0 e <sup>-104</sup>	TI/TI
		Protein phosphatase 2C (PP2C)	At4g08260	1.3 e <sup>-99</sup>	2.0 e <sup>-99</sup>	TI/TI
		Protein phosphatase 2C (PP2C)	At2g40180	3.9 e <sup>-95</sup>	5.3 e <sup>-95</sup>	PI/PI
		Calcium-dependant protein kinase (CPK1)	At5g04870	4.1 e <sup>-239</sup>	7.0 e <sup>-217</sup>	PI/NC
NiCDPK2	AJ344154	Calium-dependant protein kinase (CPK2)	At3g10660	9.1 e <sup>-233</sup>	1.5 e <sup>-209</sup>	NC/NC
		Calcium-dependant protein kinase (CPK20)	At2g38910	3.0 e <sup>-212</sup>	1.5 e <sup>-166</sup>	NC/NC

<sup>a</sup>Summary of the *AtACRE* transcription patterns in Ler treated suspension cells and Col-0 treated seedlings. TI, transiently induced; PI, progressively induced; NC, no change.

In the nonhost interaction, we found that 12% of the genes induced after 3 hpi with *Psp* overlap with *flg22*-induced genes from both *Arabidopsis* elicited seedlings and cell cultures (Table V). Similar analysis at the 6 hpi timepoint revealed a more substantial overlap of 34% commonly induced genes between *FLARE* genes and genes induced by *Psp* bacterial treatment (Table VI). Highlights of these genes include 5 members of WRKY transcription factors, 16 receptor-like kinases, and 9 genes involved in the production of ROS (see Supplemental Table XVI). Although we did not have any data with *hrp* mutants from *Psp*, the majority of these genes might be induced in a PAMP dependent manner (Jakobek and Lindgren, 1993; Lu et al., 2001).

The analysis of genes induced in compatible interactions revealed a much smaller overlap with the *FLARE* gene set than did the nonhost interaction. Indeed, only 7% of genes were commonly induced upon *flg22* treatment and in 6 hpi with compatible *Pst* (Tables V and VI). Because *flg22* peptide derived from

*P. syringae* pv *tomato* is a potent elicitor of defense responses in *Arabidopsis* (data not shown), this result suggests that some type III secretion proteins from *Pst* are potentially involved in repressing the flagellin-mediated response. To identify potential targets of these type III suppressor proteins, we selected genes that were both *flg22*- and *Psp*-induced but not up-regulated in *Pst* compatible interactions at 6-hpi timepoint. From this gene list, we also subtracted genes that were still induced in *P. syringae* pv *maculicola* at the same timepoint (data not shown). This allows the identification of candidates targeted by two different *P. syringae* pathovars. These genes are potentially involved in the nonhost resistance phenomenon observed in the *Arabidopsis*-*Psp* interaction. As a result of this analysis, we discovered 77 candidate genes including 11 transcription factors and 8 receptor-like kinases as examples (Table VII; see Supplemental Table XVII). Of these, 2 glycosyl-hydrolases (At3g13790, At3g54420) might be involved in cell wall synthesis, which is in agreement with recent report



**Figure 4.** Temporal expression patterns of Arabidopsis *ACRE* orthologs. Semiquantitative RT-PCR transcript profiling of *AtACRE* genes of *Ler* suspension cells (A) and Col-0 seedlings (B) challenged with  $\pm$ flg22 peptide for 0, 30, and 60 min and for 0, 10, 30, and 60 min, respectively. *AtACRE* genes (from top to bottom): *AtACRE1a* (At5g47230), *AtACRE1b* (At4g17490), *AtACRE4* (At5g17680), *AtACRE31* (At4g20780), *AtACRE74a* (At5g37490), *AtACRE74b* (At1g66160), *AtACRE111* (At4g25470), *AtACRE126* (At5g15130), *AtACRE132* (At3g16720), *AtACRE189* (At5g67250), *AtACRE231* (At3g28340), *AtACRE264a* (At2g05940), *AtACRE264b* (At5g35580), *AtACRE276* (At1g29340), *AtACRE284a* (At2g30020), *AtACRE284b* (At4g08260), *AtACRE284c* (At2g40180), and *AtCPK1* (At5g04870). RT-PCR of a constitutively expressed actin gene (At5g09810) was performed to control equal cDNA amount in each reaction (bottom lane).

suggesting that *P. syringae* type III effectors might suppress cell wall based plant defense 12 hpi with virulent *Pst* DC3000 (Hauck et al., 2003).

We also identified a 1-aminocyclopropane-1-carboxylate synthase, termed *AtACS6* gene (At4g11280), which represents a key component of ethylene biosynthesis together with the ethylene responsive transcription factor *AtERF5* (At5g47230), suggesting that *Pst* might suppress some ethylene-related genes (see Supplemental Table XVII in information).

Moreover, of the three RING zinc finger genes that were induced upon both *Psp* and flg22 treatments, none was induced 6 hpi with either *Pst* or *Psm* treatments (Table VII; Supplemental Table XVII). This result is

consistent with the involvement of protein turnover components in nonhost resistance (Peart et al., 2002).

Interestingly, although not present on this array, the nonhost resistance gene *NHO1* (At1g80460) is induced in Arabidopsis elicited cell cultures (data not shown) and the expression of this gene is also suppressed 6 hpi with *Pst* strain (Kang et al., 2003). Thus, this gene represents an internal control for the identification of potential targets of type III suppressor proteins.

Among the 77 candidate genes mentioned, 35 were induced specifically in interactions involving *AvrB* or *AvrRpt2* with the cognate R gene, suggesting that the R-gene/*Avr*-gene interaction negates the suppression effect mediated by virulent bacteria as suggested for *NHO1* gene (Kang et al., 2003).

In more general terms, we found that approximately 45% of the *FLARE* genes were also induced 3 hpi with *Pst* carrying either *AvrB* or *AvrRpt2* (Table V; Supplemental Table XV). Of these, approximately 30% are induced in an *AvrB*- or *AvrRpt2*-specific manner, based on *Pst* (*AvrB*) versus *Pst* and *Pst* (*AvrRpt2*) versus *Pst* comparisons (Table V; Supplemental Table XVI). This result suggests that *Avr* effector proteins might trigger a common gene subset very early after race-specific elicitor recognition and therefore enhance the PAMP-mediated innate immune response. At 6-hpi timepoint, we observed a decrease in the overlap between *FLARE* genes and genes up-regulated by *AvrB* and *AvrRpt2* race-specific elicitors; only approximately 25% of overlap was found between the flg22-induced genes and genes induced by either *AvrB* or *AvrRpt2* (Table VI). In addition, only approximately 20% of the *FLARE* genes were induced at 9 hpi of either *Pst* (*AvrB*) or *Pst* (*AvrRpt2*; data not shown). This last result suggests that the flg22 response and the *AvrB*/*AvrRpt2*-mediated defense responses might diverge at later timepoints explaining the different outcomes between these responses such as cell death in *AvrB*/*AvrRpt2*- but not in flg22-induced defense.

#### Effects of a Cycloheximide Treatment on *FLARE* Gene Expression in Arabidopsis Seedlings

The protein synthesis inhibitor CHX was used to assess whether the *FLARE* genes require de novo protein synthesis for their transcriptional activation. Arabidopsis seedlings were treated for 30 min with CHX prior to a 30-min treatment with flg22 peptide (see "Materials and Methods" for details). Transcriptional changes were then monitored by microarray and similar criteria were used to select differentially expressed genes as described before (see "Materials and Methods" for details). We found that approximately 70% of the overall *FLARE* genes displayed similar transcriptional changes in CHX/flg22 treated seedlings (see Supplemental Table XVIII). Moreover, by taking the *FLARE* induced genes as a baseline, we found that approximately 92% of the flg22-induced genes are up-regulated upon both CHX and flg22 (see Supplemental Table XIX). This result suggests that

**Table IV.** Frequency of occurrence of conserved binding motifs for different types of transcription factors in the cluster containing *AtACRE31* ortholog

Transcription Factor Type	Motif Sequences	Frequency in flg22-Regulated Promoters (48 Promoters)	Frequency in Non-flg22-Regulated Promoters (500 Promoters)	Frequency Fold Change
AP2/EREBP (GCC-box)	GCCGCC	0.10	0.08	1.25
AP2/EREBP	ACCGCC	0.10	0.09	1.11
Myb	G(G/T)T(AT)G(G/T)T	2.10	1.40	1.50
bZIP (TGA-type)	TGACG	1.27	0.88	1.44
bZIP (GBF-type)	CACGTG	0.20	0.15	1.33
bZIP (G/HBF-1 type)	CCTACC	0.12	0.12	–
EIN3/EIL	GGATGTA	0.06	0.04	1.5
WRKY (core)	TTGAC	4.10	2.05	<b>2.0</b>
WRKY (stringent)	TTGAC(T/C)	2.35	1.09	<b>2.35</b>
WRKY (stringent)	TTGACT	1.6	0.7	<b>2.3</b>
WRKY (stringent)	TTGACC	0.75	0.42	1.78
WRKY (stringent)	TTGACTT	0.69	0.28	<b>2.46</b>
W like	TTTGACA	0.60	0.30	<b>2.0</b>

In bold are the frequencies of over-representative elements that are at least twice the statistical expected frequency that occur within a set of 500 non-flg22 regulated promoters.

new protein synthesis is not required to induce the vast majority of the *FLARE* genes. On the contrary, the analysis of nonoverlapping genes revealed approximately 70% of genes predicted to be repressed by flg22 (see Supplemental Table XX). This observation suggests that the majority of flg22-repressed genes require de novo protein for their transcriptional inactivation.

Interestingly, when *Arabidopsis* seedlings were treated with CHX alone, 82% of the *FLARE* genes were induced (see Supplemental Table XIX). This result is consistent with the transcriptional activation of a large set of *ACRE* genes in Cf-9-tobacco cell culture challenged with CHX for 30 min (Durrant et al., 2000) and suggests that *FLARE* and *ACRE* genes are negatively regulated by rapidly turned over repressor proteins. It also confirms the key role played by protein turnover in the initiation of the plant defense response and suggests that relief of negative regulation is important to activate plant defense.

## DISCUSSION

The innate immune response mediated by pathogen molecules, also referred to as PAMPs is shared between plants and mammals (Gómez-Gómez and Boller, 2002; Nürnberger and Brunner, 2002). In plants, the PAMP perception activates defense responses and so far little is known about the interplay between the PAMP response and compatible/incompatible plant/pathogen interactions. To address this we performed expression profiling of *Arabidopsis* cell cultures and seedlings challenged with flg22. We identified many components involved in signaling. Clustering analysis revealed three main groups of coregulated *FLARE* genes. A subset of progressively induced *FLARE* genes contains an over-representation of the W-box element and a W-box-like element within their promoters. The *FLARE* gene set was then compared to the set of *ACRE*

genes previously identified as induced in Cf9-tobacco cell cultures challenged with the fungal derived Avr9 peptide. This revealed a substantial overlap between the *FLARE* and *ACRE* gene induction and highlights common defense processes shared between the bacterial PAMP response and fungal race-specific defense responses.

To further analyze the cross-talk between flg22-innate immune response, nonhost interaction, gene-for-gene, and compatible interactions, we compared our set of *FLARE* genes with genes up-regulated in *Pst*, *Pst* carrying either *AvrB* or *AvrRpt2*, and *Psp* inoculations. This comparative analysis suggests that (1) the flagellin response is likely to mimic nonhost defense responses, (2) *Pst* might suppress the expression of genes potentially involved in nonhost resistance as well as gene-for-gene resistance, and (3) incompatible

**Table V.** Overlap between *FLARE* genes and genes induced after 3 hpi of different bacterial treatments

Treatments	cFLARE		sFLARE	All FLARE Genes
	30 min	60 min	30 min	
<i>Pst</i>	12	14	8	8
<i>Pst</i> ( <i>AvrB</i> )	63	64	65	49
<i>Pst</i> ( <i>AvrB</i> ) vs <i>Pst</i>	48	39	51	35
<i>Pst</i> ( <i>AvrRpt2</i> )	55	54	60	44
<i>Pst</i> ( <i>AvrRpt2</i> ) vs <i>Pst</i>	34	23	41	25
<i>Psp</i>	21	21	14	12

Percentage distribution of *FLARE* genes that are commonly regulated in compatible (*Pst*), incompatible (*Pst* (*AvrB*), *Pst* (*AvrRpt2*), *Pst* (*AvrB*) vs *Pst*, *Pst* (*AvrRpt2*) vs *Pst*), and non host (*Psp*) interactions (compared to Tao et al., 2003). cFLARE genes signifies genes induced in cell cultures (30-min and 60-min timepoints); sFLARE genes signifies genes induced in seedlings (30-min timepoint); all *FLARE* genes signifies genes induced either in cell cultures (30-min and 60-min timepoints) or in seedlings (30-min timepoint).

**Table VI.** Overlap between *FLARE* genes and genes induced after 6 hpi of different bacterial treatments

Treatments	Cell Cultures		Seedlings	<i>FLARE</i> Genes
	30 min	60 min	30 min	
<i>Pst</i>	8	8	6	7
<i>Pst</i> ( <i>AvrB</i> )	48	49	38	34
<i>Pst</i> ( <i>AvrB</i> ) vs <i>Pst</i>	36	42	29	27
<i>Pst</i> ( <i>AvrRpt2</i> )	40	39	41	32
<i>Pst</i> ( <i>AvrRpt2</i> ) vs <i>Pst</i>	28	25	30	23
<i>Psp</i>	43	47	43	34

Percentage distribution of *FLARE* genes that are commonly regulated in compatible (*Pst*), incompatible (*Pst* [*AvrB*], *Pst* [*AvrRpt2*], *Pst* [*AvrB*] vs *Pst*, *Pst* [*AvrRpt2*] vs *Pst*), and non host (*Psp*) interactions (compared to Tao et al., 2003). *cFLARE* genes signifies genes induced in cell cultures (30-min and 60-min timepoints); *sFLARE* genes signifies genes induced in seedlings (30-min timepoint); all *FLARE* genes signifies genes induced either in cell cultures (30-min and 60-min timepoints) or in seedlings (30-min timepoint).

interactions mediated by either *AvrB* or *AvrRpt2* might negate this suppression effect and thus promote resistance. We also identified potential targets for *P. syringae* pv *tomato* and *maculicola* suppressor type III proteins.

#### Highlights of *FLARE* Genes and Their Potential Role in Signaling Transduction

Treatment of Arabidopsis cell cultures and seedlings with flg22 elicitor results in the differential regulation of 3% of 8,200 genes within 60 min. None of these genes was induced or repressed in an *fls2-17* seedling mutant after flg22 treatment (Zipfel et al., 2004). Many induced genes encode signaling components, including transcription factors, protein kinases, and phosphatases and proteins that regulate protein turnover. Reversible phosphorylation is likely to play a role in the activation and inactivation of MAP kinases (MAPKs) in signaling pathways triggered by elicitors and stress signals. The identification of *FLARE* genes coding for protein phosphatase 2C suggests a possible role for these proteins as negative regulators of the flg22-activated MAPK cascade (Asai et al., 2002).

An interesting feature of the flg22/FLS2 response is the repression of auxin signaling-related genes in Arabidopsis treated cell cultures and seedlings, including genes encoding Aux/IAA proteins. Aux/IAA proteins were first isolated as members of a gene family that is rapidly induced in response to auxin (Abel et al., 1994). Upon flg22 treatment, the rapid repression of these auxin-related genes might contribute to the growth inhibition observed in flg22-treated Arabidopsis seedlings (Gómez-Gómez et al., 1999).

#### Involvement of Protein Degradation in the Plant Defense Response

Among the *FLARE* genes, several genes potentially involved in protein degradation were identified. In the

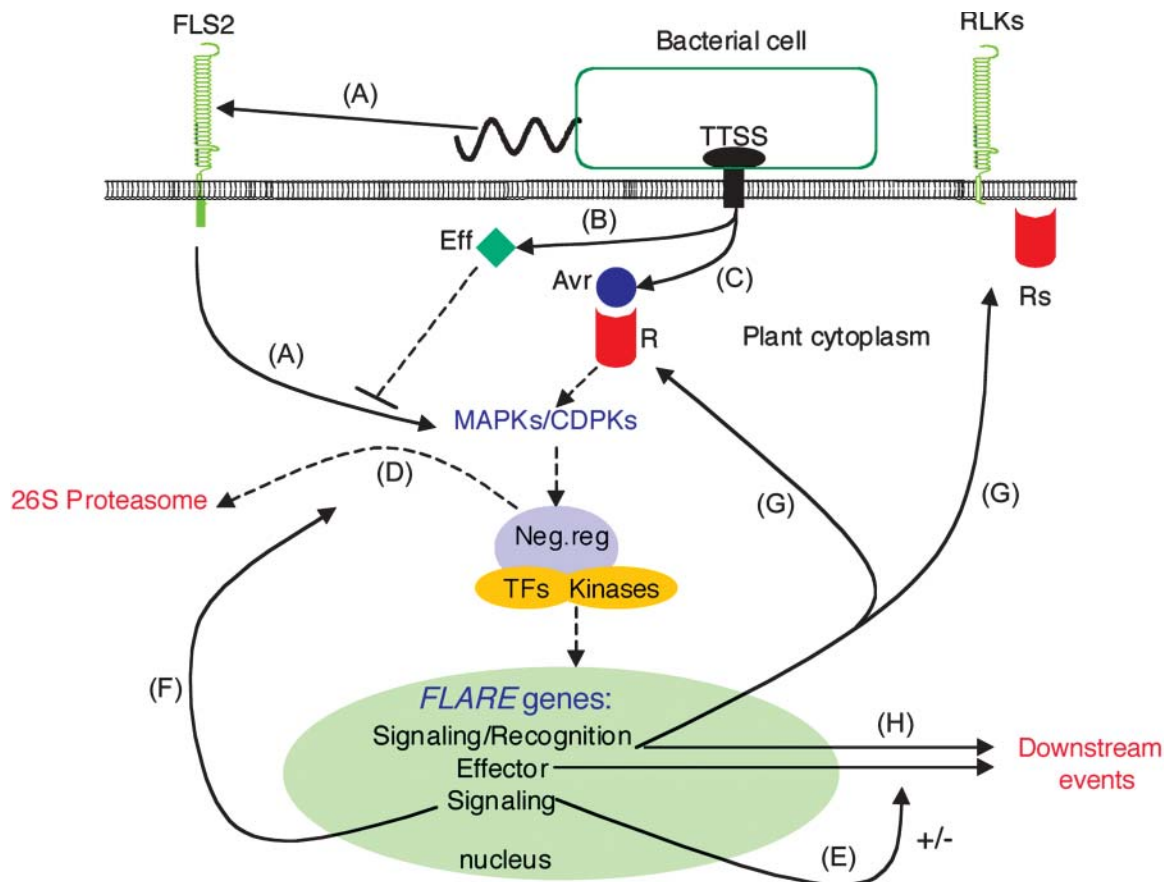
early innate immune response in mammals, the proteolytic degradation of I $\kappa$ B via the proteasome leads to the translocation of the NF- $\kappa$ B transcription factors to the nucleus to activate transcription (Karin and Ben Neriah, 2000; Read et al., 2000; Silverman and Maniatis, 2001). In plant defense signaling, SGT1, an SCF-complex-associated protein, is required for protein turnover in the auxin response (Austin et al., 2002; Azevedo et al., 2002; Gray et al., 2002, 2003; Peart et al., 2002). In the auxin response, SCF<sup>TIR1</sup> and related SCF complexes bind Aux/IAA proteins, leading to their degradation (Gray et al., 2001). *Aux/IAA* genes were reported to be induced upon CHX treatment, which is presumed to induce genes by preventing translation of mRNAs encoding rapidly turned over repressor proteins (Abel et al., 1995). Similarly, the transcriptional activation of the majority of *FLARE* genes upon CHX treatment suggests that accelerated proteolysis of repressors might be involved in activation of the plant immune response (see Supplemental Table XVIII). Such proteins are not necessarily direct transcriptional repressors; they could include other kinds of negative regulators of defense mechanisms.

Upon flg22 treatment, 10 genes encoding RING zinc-finger proteins were significantly induced (Table I). Such proteins are thought to have an E3-ligase activity and previous studies revealed their involvement in the elicitor response (Salinas-Mondragon et al., 1999; Takai et al., 2002). We also found induction of the U-box proteins *AtPUB5*, 12, 17 (*AtACRE276*), and 20/21 (*AtACRE74*) upon flg22 treatment (Table I; Fig. 4). These genes encode proteins with a conserved U-box domain, which structurally resembles the RING finger domain (Aravind and Koonin, 2000; Ohi et al., 2003). In addition, we observed flg22 inducibility of a

**Table VII.** Summary table displaying the proportion of genes potentially targeted by *Pst* and *Psm* type III secreted proteins

Group	Function	<i>FLARE/Psp</i>	<i>FLARE/Psp</i>
		Induced Genes Total Number	Induced Genes Minus <i>Pst/Psm</i> Induced Genes
Effector	Cell wall modification	8	<b>2</b>
	Hormone signalling	9	<b>5</b>
	Secondary product	5	<b>2</b>
	Ion responsive	6	<b>6</b>
	Kinase/Phosphates	4	<b>3</b>
Signaling	Protein turnover	3	<b>3</b>
	ROS production	9	<b>5</b>
	Transcription factors	19	<b>11</b>
Signaling/ recognition	Receptor-like kinases	16	<b>8</b>
	Resistance-related	3	<b>0</b>
Miscellaneous	Others	19	<b>15</b>
	Unknown	21	<b>16</b>

In bold are the number of genes in each functional category that are potentially targeted by *Pst* and *Psm* type III secreted proteins.



**Figure 5.** Model for the role of *FLARE* and *ACRE* genes in early plant defense processes. Dashed arrows indicate hypothetical processes. Plain arrows indicate the role that *FLARE* genes are likely to play according to our current survey and previous studies in plant defense signaling. Neg. reg., TFs, TTSS, Eff, and Avr stand for negative regulator of defense, transcription factor, type III secretion system, virulent bacterial effector protein, and avirulent protein, respectively.

putative ortholog of the tobacco *ACRE189* gene termed *SKIP2* (At5g67250), which encodes an F-box protein with LRR domains. F-box proteins are components of the E3-ligase SCF complex and are involved in the delivery of appropriate targets to this complex for ubiquitylation followed by degradation in the proteasome (Deshaies, 1999; Kipreos and Pagano, 2000). Several negative regulators of plant defense responses have been previously reported (Dietrich et al., 1997; Li et al., 1999; Clough et al., 2000). As an example, *edr1* (*enhanced disease resistance*) was found to enhance disease resistance to the fungus *Erysiphe cichoracearum* (Frye and Innes, 1998). In addition, *SNI1* (*suppressor of npr1-1, inducible 1*) was found to suppress mutations in *NIM1/NPR1*, a positive regulator of the general plant defense systemic acquired resistance response (Li et al., 1999). These genetic studies suggest that the plant immune response is under negative regulation. Such negative regulators might be the targets of the *FLARE/ACRE* genes involved in 26S-proteasome pathways similar to the degradation of I $\kappa$ B, a negative regulator of NF- $\kappa$ B transcription factor, in animal systems. The identification of such putative negative regulators is a high priority for future studies.

### Repertoire of *RLK/R FLARE* Genes and Their Potential Role in Resistance

We identified several resistance genes, putative resistance genes and *RLK* genes that are induced upon flg22 treatment. These genes were classified as signaling-perception-related genes (Table II). The *RLKs* belong to various subclasses according to their extracellular domains and are likely involved in recognition of extracellular signals. For example, we found an *RLK* with a lysin extracellular domain (At2g33580). This conserved motif was originally identified in bacteria and is thought to function in general peptidoglycan binding (Ponting et al., 1999; Bateman and Bycroft, 2000). Elevated mRNA levels of genes encoding *RLKs* suggest that flg22 may enhance the sensitivity of plant cells to many different PAMPs. Therefore, the *FLARE RLK* genes are likely to represent components important for the perception of various general elicitors or even race-specific elicitors. Intriguingly, transcript elevation of several resistance genes as well as genes required for resistance were detected (Table II). Although flg22 is a bacterial PAMP, we identified *FLARE* genes coding for homologs of R proteins conferring

resistance to oomycetes, bacteria, fungi, nematodes, and viruses. So far, only the *R* gene *Xa1* was reported to be up-regulated by pathogen infection (Yoshimura et al., 1998), and none of the recent RNA profiling experiments have shown a differential expression pattern of these *R* genes (Maleck et al., 2000; Tao et al., 2003).

### Suppression of PAMP Induced Genes by Virulent *P. syringae*

Nonspecific recognition of general elicitors produced by nonhost pathogens plays a major role in the nonhost inducible defense response (Jakobek and Lindgren, 1993; Lu et al., 2001). Consistent with this, we found that 34% of the *FLARE* genes were commonly induced in Arabidopsis-*Psp* interaction 6 hpi (Table IV). Because Arabidopsis is resistant to the *Psp* nonhost strain, PAMP-mediated response might significantly contribute to this resistance phenomenon. Whereas nonhost resistance remains poorly investigated, some components have emerged. As an example, *NHO1* was identified throughout a genetic screen for reduced nonhost resistance mediated by *Psp*. This Arabidopsis gene encodes a glycerol kinase homolog that is also involved in gene-for-gene interaction (Kang et al., 2003). *NHO1* is induced by *P. syringae* pv *phaseolicola*, *P. syringae* pv *syringae*, and *P. syringae* pv *tabaci* alike, suggesting that PAMPs shared between these bacteria are responsible for induction of this gene (Kang et al., 2003). Interestingly, we found this particular gene induced in Arabidopsis cell cultures challenged with flg22 peptide (data not shown). In this study, we report that only 7% of the flg22-induced genes were also induced upon 6 hpi of *Pst* bacterial strain (Table VI). This result suggests that some type III effector proteins might suppress the flg22-innate immune response and other PAMP-triggered responses, as suggested by recent work on the HopPtoD2 effector protein (Espinosa et al., 2003). We identified 77 potential targets for these *P. syringae* pv *tomato* type III suppressors (see Supplemental Table XVII). Like *NHO1* nonhost resistance gene, these candidate genes might play a crucial role in nonhost resistance.

### Connection between PAMPs- and Race-Specific Defense Responses

The early transcriptional changes that occur in the Arabidopsis flg22/FLS2 response and the tobacco Avr9/Cf-9 responses display a striking overlap. For 13 out of 17 tobacco *ACRE* full-length cDNAs, we found that at least one representative of their orthologs was also induced in flg22-elicited suspension cells and seedlings (Table III; Fig. 4). We also identified *AtMPK3* (At3g45640) as flg22-induced (Fig. 1, B and D). This gene was reported to be involved in flg22 signaling (Nühse et al., 2000) and is orthologous to the tobacco *WIPK* gene that was rapidly induced by Avr9 peptide in Cf-9-tobacco suspension cells (Romeis et al., 2000).

In addition, we observed that a large set of *FLARE* genes were rapidly elicited after infection 3 hpi with *Pseudomonas* strains carrying *AvrB* and *AvrRpt2* avirulence genes (Table V; Supplemental Table XV). Such overlap in response to a race-specific elicitor and a general elicitor highlights a conserved process of plant immunity and suggests that other pathogen-derived elicitors induce similar subsets of genes through different receptors. Moreover, this overlap suggests that race-specific resistance triggered by specific Avr genes may have evolved from mechanisms involved in recognition of PAMPs. Since plants lack mechanisms of acquired immunity, the evolution of polymorphism in recognition capacity for multiple pathogen-derived molecules could have led to the gene-for-gene interactions that we observe today (Dangl and Jones, 2001). Further investigation on the specificity of flg22/FLS2 and Avr9/Cf-9 transcript signatures will provide clues to explain the different outcomes of these responses such as the cell death observed in the Avr9-race-specific defense response, but not in flg22 innate immune response.

### Model for Early Signaling Events in Arabidopsis Bacterial Response

We present here a model showing the interplay between flg22-triggered innate immune and early virulent and avirulent bacterial responses (Fig. 5). When potentially pathogenic *P. syringae* strains enter plant tissue, their PAMPs (such as flagellin) can elicit defenses through FLS2 and other receptors (arrow A). To suppress this elicitation, effector proteins are delivered into host cells through the type III secretion system (arrow B). In an incompatible interaction, some effector proteins (that can be recognized genetically as Avr proteins) interact with complexes containing host R proteins and elicit the defense response through R gene-dependent recognition (arrow C). This elicitation could occur through mechanisms that involve the central positive regulators of defense such as MAPKs or CDPKs that were targeted by the bacterial effector proteins.

After recognition, both race-specific and PAMP elicitors trigger similar responses such as ion fluxes, production of ROS, and activation of MAPKs and CDPKs (Felix et al., 1999; Gómez-Gómez et al., 1999; Grant et al., 2000; Asai et al., 2002). flg22 (and presumably other PAMP) elicitation leads to rapid and transient induction of signaling-related genes presumably through degradation of negative regulators of defense such as transcription factors and kinases (arrow D). The *FLARE* genes encoding proteins involved in protein turnover, such as RING finger and U-box proteins, are likely to be involved in ubiquitination of these negative regulators of defense (arrow F). Other induced signaling-related genes trigger the induction or repression of downstream components (arrow E). The progressively induced transcripts contain *RLKs* as well as some *R* genes, and point to a possible interaction between the innate immune

response mediated by PAMPs and sensitization of the cells for further pathogen recognition (arrow G). Other progressively induced transcripts encode components that might be involved more directly in plant defense processes such as antimicrobial proteins (arrow H).

Overall, then, these data suggest that PAMPs such as flagellin play an important role in plant/pathogen interactions. Their existence has led to selection for a large set of bacterial effector proteins that suppress PAMP-elicited pathways. PAMP elicitation leads to elevated levels of R proteins and of receptors for PAMPs. This complex evolutionary interplay still provides fertile ground for exciting new insights into the mechanisms that are involved.

## MATERIALS AND METHODS

### Cell Culture Materials and Elicitor Treatment

Cell cultures of *Arabidopsis Ler* were maintained and used for analysis 7 d after subculturing as previously described (Felix et al., 1999). The pH of the cell cultures was measured with a small combined glass electrode (Metrohm, Basel). Elicitor peptide flg22 was synthesized by Sigma Genosys (St. Louis) diluted in dimethyl sulfoxide solvent and added to a concentration of 100 nM 75 min after transferring an aliquot of the cell cultures to a beaker on a rotary shaker. Cells were harvested by filtration, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$ . Cells of *Arabidopsis Col-0* (Ferrando et al., 2000) were used 4 d after subculture and similar flg22 treatments were applied.

### Seedling Materials and Treatments

After a 48-h treatment at  $4^{\circ}\text{C}$ , *Arabidopsis Col-0* seeds were grown for 12 d on plates containing  $1 \times$  Murashige and Skoog medium (Duchefa), 1% Suc, and 1% agar under continuous light conditions of  $60 \mu\text{E m}^{-2} \text{s}^{-1}$  at  $22^{\circ}\text{C}$ . Seedlings were then transferred to liquid Murashige and Skoog medium (2 seedlings/500  $\mu\text{L}$  of medium in wells of 24-well-plates). Two days after transfer the medium was supplied with flg22 peptide to a final concentration of 10  $\mu\text{M}$ . Plantlets were collected 30 min after treatment, frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . In the case of the CHX experiment, 50  $\mu\text{M}$  CHX was added 30 min prior to flg22 or water treatment.

For assaying ethylene production, 2-week-old seedlings, grown in liquid Murashige and Skoog medium, were transferred to 6-mL glass tubes (2 seedlings/tube) containing 1 mL of an aqueous solution of 10  $\mu\text{M}$  flg22. Vials were closed with rubber septa and ethylene accumulating in the free air was measured by gas chromatography.

### RNA Preparation and Microarray Processing

For cell cultures, total RNA was extracted using Trizol-Reagent (Sigma). RNA samples were cleaned over Qiagen RNeasy mini-columns (Valencia, CA). For seedlings, total RNA was extracted using RNeasy Plant Mini kit (Qiagen). Genome arrays, washing, staining, and scanning were carried out according to the manufacturer's suggestions (Affymetrix).

### Transcript Profiling of ACRE Orthologs by RT-PCR

Total RNA from two independent cell culture experiments were extracted as described previously and pooled. Two micrograms of DNase-treated RNA were reverse transcribed for 90 min at  $42^{\circ}\text{C}$  in a 20- $\mu\text{L}$  reaction volume containing 1 unit of SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA), 250  $\mu\text{M}$  each dNTP, 30  $\mu\text{M}$  oligo(dT) 30 M primer, 20 units of RNase inhibitor, and 10 mM dithiothreitol. One microliter of the RT reaction was used for PCR in a 20- $\mu\text{L}$  volume with 1 unit of Taq DNA-polymerase (Qiagen), 100  $\mu\text{M}$  each dNTP, and 100 ng of each forward and reverse primers from *AtACRE* genes. PCR conditions were the following: 3 min,  $94^{\circ}\text{C}$  (first cycle); 30 s,  $94^{\circ}\text{C}$ ; 30 s,  $50^{\circ}\text{C}$ ; 1.5 min,  $72^{\circ}\text{C}$  (24–27 cycles); and 10 min,  $72^{\circ}\text{C}$  (last cycle). PCR products were separated on a 1% agarose gel and visualized after ethidium bromide staining. To control equal cDNA amount in each reaction, a PCR was performed with primers corresponding to the actin gene (*At5g09810*),

which is constitutively expressed in vegetative structures *AC1* (5'-ATGGCA-GACGGTGAGGATATTCA-3') and *AC2* (5'-GCCTTGCAATCCACATCT-GTTTG-3').

### Identification of FLARE Genes

Genes were considered as up- or down-regulated if their expression level in elicited *Ler* cell culture deviated (positively or negatively) more than 2.5-fold from that of the unelicited *Ler* cell cultures in both independent experiments and if the genes were called I for increase and D for decrease as a result of the statistical comparative analysis performed using Microarray Suite Software MAS4 (Affymetrix). Before applying this filter, genes with an expression level above 10 (noise level of expression) were previously selected. For the *Col-0* seedling assay, similar criteria were used to select flg22-regulated genes and the statistical analysis were performed using MAS5 (Affymetrix). To generate the list of *FLARE* genes with their appropriate annotation, the Affymetrix probe set-IDs for the flg22-regulated genes were collected and used to retrieve annotation and AGI numbers from the Salk Institute Genomic Analysis Laboratory database SIGnAL (<http://signal.salk.edu/about.htm>). Alternatively, when gene annotations were not found, their corresponding cDNA sequences were collected using the Julian Schroeder's database (<http://www.biology.ucsd.edu/labs/schroeder/trendsreview.html>) and searched against TIGR (<http://tigrblast.tigr.org/er-blast/index.cgi?project=ath1>) as well as the MIPS ([http://mips.gsf.de/proj/thal/db/search/blast\\_arabi.html](http://mips.gsf.de/proj/thal/db/search/blast_arabi.html)) *Arabidopsis* databases using a BLASTN program (Altschul et al., 1997). Additional annotations were identified from the ones associated with probe sets on the Affymetrix chip. Receptor-like kinases were classified according to the identity of the extracellular domains (Shiu and Bleecker, 2001), and the extracellular domain of each nonpreclassified RLK was identified using the SMART database ([http://smart.embl-heidelberg.de/help/smart\\_about.shtml](http://smart.embl-heidelberg.de/help/smart_about.shtml)).

### Comparative Analysis between Flare Genes and Genes Induced by Different Bacterial Treatments

Raw data derived from samples treated for 3 hpi and 6 hpi of water, *P. syringae* pv *tomato* (*Pst*), *P. syringae* pv *tomato* carrying either AvrB or AvrRpt2, and *P. syringae* pv *phaseolicola* (*Psp*) were used for analysis (Tao et al., 2003). Average from expression level of each probe set of a treatment was calculated. To select genes up-regulated in compatible interaction, average expression level from each probe set at each timepoint was divided by average expression level of the water treated samples at the corresponding timepoint. Similar selection was performed for the identification of genes induced in nonhost interaction mediated by *Psp*. For the identification of genes induced in incompatible interactions, average expression level from each probe set at each timepoint was divided by either average expression level of the water treated samples or *Pst* treated samples at each timepoint. This last selection allows the identification of genes specifically induced upon race-specific elicitors AvrB or AvrRpt2. Genes that deviate positively more than 2.5-fold change were then selected as significantly induced and compared to the flg22-induced genes derived from elicited cell cultures and seedlings. Moreover, we selected only probe sets with expression level equal or above 10 (noise level of expression). Similar selection criteria were used to identify flg22-induced genes.

### Data Processing and Data Analysis

Global analysis of temporal gene expression pattern was performed by subjecting the absolute expression values of the overall *FLARE* genes over the time course to a SOM algorithm using  $3 \times 1$  two-dimensional matrix with default SOM filters (DMT, Affymetrix). The sequences of the 5' regions (up to 1,100 bp) were used to search for sequences (5–8 bp) that are over-represented within the progressively induced cluster (*AtACRE31* regulon) and the transiently induced cluster (*AtACRE1* regulon containing *AtACRE111/132/264*) compared with all genes outside of these clusters. This motif search algorithm was performed using GENESPRING software and only oligomers with *P* values below 0.05 cutoff were considered as significantly over-represented. For further promoter analysis, we extracted 1-kb promoter sequences from TAIR database (<http://www.arabidopsis.org/tools/bulk/sequences/index.html>) and analyzed the over-representation of this regulatory elements according to Maleck et al., 2000).

Sequence data from this article have been deposited with the EMBL/GenBank data libraries under accession numbers AF211527, AF211528, AF211529, AY220484, AF211530, AY220477, AF211532, AF211537, AY220478, AY220479, AY220480, AF211536, AY220481, AY220482, AY220483, AY220484, and AJ344154.

## ACKNOWLEDGMENTS

We thank J. Hadfield (JIC) and E. Oakeley (FMI) for help in the array procedure and analysis. We thank S. Peck for help throughout this work. We also thank K. Bouarab and Corbier for comments on the manuscript.

Received November 25, 2003; returned for revision February 9, 2004; accepted February 11, 2004.

## LITERATURE CITED

- Abel S, Oeller PW, Theologis A (1994) Early auxin-induced genes encode short-lived nuclear proteins. *Proc Natl Acad Sci USA* **91**: 326–330
- Abel S, Nguyen MD, Theologis A (1995) The PS-IAA4/5-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. *J Mol Biol* **251**: 533–549
- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402
- Aravind L, Koonin EV (2000) The U-box is modified RING finger: a common domain in ubiquitination. *Curr Biol* **10**: R132–R134
- Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gómez-Gómez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* **415**: 977–983
- Austin MJ, Muskett P, Kahn K, Feys BJ, Jones JDG, Parker JE (2002) Regulatory role of SGT1 in early R gene-mediated plant defenses. *Science* **295**: 2077–2080
- Axtell MJ, Staskawicz BJ (2003) Initiation of *RPS2*-specified disease resistance in *Arabidopsis* is coupled to the *AvrRpt2*-directed elimination of RIN4. *Cell* **112**: 369–377
- Azevedo C, Sadanandom A, Kitagawa K, Freialdenhoven A, Shirasu K, Schulze-Lefert P (2002) The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. *Science* **295**: 2073–2076
- Bateman A, Bycroft M (2000) The structure of a LysM domain from *E. coli* membrane-bound lytic murein transglycosylase D (MltD). *J Mol Biol* **299**: 1113–1119
- Boch J, Joardar V, Gao L, Robertson TL, Lim M, Kunkel BN (2002) Identification of *Pseudomonas syringae* pv. *tomato* genes induced during infection of *Arabidopsis thaliana*. *Mol Microbiol* **44**: 73–88
- Clough S, Fengler K, Yu I-C, Lippok B, Smith R, Bent A (2000) The *Arabidopsis dnd1* “defense, no death” gene encodes a mutated cyclic nucleotide-gated ion channel. *Proc Natl Acad Sci USA* **97**: 9323–9328
- Collmer A, Lindeberg M, Petnicki-Ocwieja T, Schneider DJ, Alfano JR (2002) Genomic mining type III secretion system effectors in *Pseudomonas syringae* yields new picks for all TTSS prospectors. *Trends Microbiol* **10**: 462–469
- Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. *Nature* **411**: 826–833
- Dangl JL, Ritter C, Gibbon MJ, Mur LA, Wood JR, Goss S, Mansfield J, Taylor JD, Vivian A (1992) Functional homologs of the *Arabidopsis* RPM1 disease resistance gene in bean and pea. *Plant Cell* **4**: 1359–1369
- Deshaies RJ (1999) SCF and cullin/RING H2-based ubiquitin ligases. *Annu Rev Cell Dev Biol* **15**: 435–467
- Dietrich RA, Richberg MH, Schmidt R, Dean C, Dangl JL (1997) A novel zinc finger protein is encoded by the *Arabidopsis* *LSDI* gene and functions as a negative regulator of plant cell death. *Cell* **88**: 685–694
- Durrant WE, Rowland O, Piedras P, Hammond-Kosack KE, Jones JDG (2000) cDNA-AFLP reveals a striking overlap in race-specific resistance and wound response gene expression profiles. *Plant Cell* **12**: 963–977
- Espinosa A, Guo M, Tam VC, Fu ZQ, Alfano JR (2003) The *Pseudomonas syringae* type III-secreted protein HopPtoD2 possesses protein tyrosine phosphatase activity and suppresses programmed cell death in plants. *Mol Microbiol* **49**: 377–387
- Eulgem T, Rushton PJ, Robatzek S, Somssich IE (2000) The WRKY superfamily of plant transcription factors. *Trends Plant Sci* **5**: 199–206
- Felix G, Duran JD, Volko S, Boller T (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J* **18**: 265–276
- Ferrando A, Farras R, Jasik J, Scheel J, Koncz C (2000) Intron-tagged epitope: a tool for facile detection and purification of proteins expressed in *Agrobacterium*-transformed plant cells. *Plant J* **22**: 553–560
- Flor HH (1971) Current status of the gene-for-gene concept. *Annu Rev Phytopathol* **9**: 275–298
- Fouts DE, Abramovitch RB, Alfano JR, Baldo AM, Buell CR, Cartinhour S, Chatterjee AK, D’Ascenzo M, Gwinn ML, Lazarowitz SG, et al. (2002) Genomewide identification of *Pseudomonas syringae* pv. *tomato* DC3000 promoters controlled by the HrpL alternative sigma factor. *Proc Natl Acad Sci USA* **99**: 2275–2280
- Frye CA, Innes RW (1998) An *Arabidopsis* mutant with enhanced resistance to powdery mildew. *Plant Cell* **10**: 947–956
- Galan JE, Collmer A (1999) Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* **284**: 1322–1328
- Gómez-Gómez L, Bauer Z, Boller T (2001) Both the extracellular leucine-rich repeat domain and the kinase activity of FLS2 are required for flagellin binding and signaling in *Arabidopsis*. *Plant Cell* **13**: 1155–1163
- Gómez-Gómez L, Boller T (2002) Flagellin perception: a paradigm for innate immunity. *Trends Plant Sci* **7**: 251–256
- Gómez-Gómez L, Felix G, Boller T (1999) A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. *Plant J* **18**: 277–284
- Grant M, Brown I, Adams S, Knight M, Ainslie A, Mansfield J (2000) The *RPM1* plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death. *Plant J* **23**: 441–450
- Gray WM, Hellmann H, Dharmasiri S, Estelle M (2002) Role of the *Arabidopsis* RING-H2 protein RBX1 in RUB modification and SCF function. *Plant Cell* **14**: 2137–2144
- Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001) Auxin regulates SCF(TIR1)-dependent degradation of AUX/IAA proteins. *Nature* **414**: 271–276
- Gray WM, Muskett PR, Chuang H-W, Parker JE (2003) *Arabidopsis* SGT1b is required for SCF<sup>TIR1</sup>-mediated auxin response. *Plant Cell* **15**: 1310–1319
- Guttman DS, Vinatzer BA, Sarkar SF, Ranall MV, Kettler G, Greenberg JT (2002) A functional screen for the type III (Hrp) secretome of the plant pathogen *Pseudomonas syringae*. *Science* **295**: 1722–1726
- Hammond-Kosack KE, Tang SJ, Harrison K, Jones JDG (1998) The tomato *Cf-9* disease resistance gene functions in tobacco and potato to confer responsiveness to the fungal avirulence gene product Avr9. *Plant Cell* **10**: 1251–1266
- Hauck P, Thilmony R, He SY (2003) A *Pseudomonas syringae* type III effector suppresses cell wall-based extracellular defense in susceptible *Arabidopsis* plants. *Proc Natl Acad Sci USA* **100**: 8577–8582
- Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* **410**: 1099–1103
- Huynh TV, Dahlbeck D, Staskawicz BJ (1989) Bacterial blight of soybean: regulation of a pathogen gene determining host cultivar specificity. *Science* **245**: 1374–1377
- Innes RW, Bent AE, Kunkel B-N, Bisgrove SR, Staskawicz BJ (1993) Molecular analysis of avirulence gene *avrRpt2* and identification of a putative regulatory sequence common to all known *Pseudomonas syringae* avirulence genes. *J Bacteriol* **175**: 4859–4869
- Jakobek JL, Lindgren PB (1993) Generalized induction of defense responses in bean is not correlated with the induction of the hypersensitive reaction. *Plant Cell* **5**: 49–56
- Jakobek JL, Smith JA, Lindgren PB (1993) Suppression of bean defense responses by *Pseudomonas syringae*. *Plant Cell* **5**: 57–63
- Janeway CA Jr, Medzhitov R (1998) Introduction: the role of innate immunity in the adaptive immune response. *Semin Immunol* **10**: 349–350
- Joosten MH, Cozijnsen TJ, De Wit PJ (1994) Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. *Nature* **367**: 384–386

- Kang L, Li J, Zhao T, Xiao F, Tang X, Thilmony R, He SY, Zhou J-M (2003) Interplay of the Arabidopsis nonhost resistance gene *NHO1* with bacterial virulence. *Proc Natl Acad Sci USA* **18**: 3519–3524
- Karin M, Ben Neriah Y (2000) Phosphorylation meets ubiquitination: the control of NF- $\kappa$ B activity. *Annu Rev Immunol* **18**: 621–663
- Keen NT (1990) Gene-for-gene complementarity in plant-pathogen interactions. *Annu Rev Genet* **24**: 447–463
- Kipreos ET, Pagano M (2000) The F-box protein family. *Genome Biol* **1**: 3002.1–3002.7
- Kunkel BN, Bent AF, Dahlbeck D, Innes RW, Staskawicz BJ (1993) RPS2, an Arabidopsis disease resistance locus specifying recognition of *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. *Plant Cell* **5**: 865–875
- Li X, Zhang Y, Clarke J, Li Y, Dong X (1999) Identification and cloning of a negative regulator of systemic acquired resistance, SN1I, through a screen for suppressors of *npr1-1*. *Cell* **98**: 329–339
- Liu W-M, Mei R, Di X, Ryder TB, Hubbell E, Dee S, Webster TA, Harrington CA, Ho M-H, Bai J, et al. (2002) Analysis of high density expression microarrays with signed-rank call algorithms. *Bioinformatics* **18**: 1593–1599
- Lu M, Tang X, Zhou JM (2001) Arabidopsis *NHO1* is required for general resistance against *Pseudomonas* bacteria. *Plant Cell* **13**: 437–447
- Mackey D, Belkadir Y, Alonso JM, Ecker JR, Dangl JL (2003) Arabidopsis *RIN4* is a target of the type III virulent effector *AvrRpt2* and modulates RPS2-mediated resistance. *Cell* **112**: 379–389
- Mackey D, Holt BE, Wiig A, Dangl JL (2002) *RIN4* interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated disease resistance in Arabidopsis. *Cell* **108**: 379–389
- Maleck K, Levine A, Eulgem T, Morgan A, Schmid J, Lawton KA, Dangl JL, Dietrich RA (2000) The transcriptome of Arabidopsis thaliana during systemic acquired resistance. *Nat Genet* **26**: 403–410
- Nühse TS, Peck SC, Hirt H, Boller T (2000) Microbial elicitors induce activation and dual phosphorylation of the Arabidopsis thaliana MAPK 6. *J Biol Chem* **275**: 7521–7526
- Nürnberg T, Brunner F (2002) Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecular patterns. *Curr Opin Plant Biol* **5**: 318–324
- Ohi MD, Vander Kooi CW, Rosenberg JA, Chazin WJ, Gould KL (2003) Structural insights into the U-box, a domain associated with multi-ubiquitination. *Nat Struct Biol* **10**: 250–255
- Pearl JR, Lu R, Sadanandom A, Malcuit I, Moffett P, Brice DC, Schausser L, Jaggard DA, Xiao S, Coleman MJ, et al. (2002) Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. *Proc Natl Acad Sci USA* **99**: 10865–10869
- Petnicki-Ocwieja T, Schneider DJ, Tam VC, Chancey ST, Shan L, Jamir Y, Schechter LM, Janes MD, Buell CR, Tang X (2002) Genomewide identification of proteins secreted by the Hrp type III protein secretion system of *Pseudomonas syringae* pv. *tomato* DC3000. *Proc Natl Acad Sci USA* **99**: 7652–7657
- Ponting CP, Aravind L, Schultz J, Bork P, Koonin EV (1999) Eukaryotic signalling domain homologues in archaea and bacteria. Ancient ancestry and horizontal gene transfer. *J Mol Biol* **289**: 729–745
- Read MA, Brownell JE, Gladysheva TB, Hottelet M, Parent LA, Coggins MB, Pierce JW, Podust VN, Luo RS, Chau V, et al. (2000) Ned8 modification of Cul-1 activates SCF <sup>$\beta$ TrCP</sup>-dependent ubiquitination of I $\kappa$ B $\alpha$ . *Mol Cell Biol* **20**: 2326–2333
- Robatzek S, Somssich IE (2002) Targets of AtWRKY6 regulation during plant senescence and pathogen defense. *Genes Dev* **16**: 1139–1149
- Romeis T, Piedras P, Jones JDG (2000) Resistance gene-dependent activation of a calcium-dependent protein kinase in the plant defense response. *Plant Cell* **12**: 803–816
- Romeis T, Piedras P, Zhang S, Klessig DE, Hirt H, Jones JDG (1999) Rapid *Avr9*- and *Cf-9*-dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound, and salicylate responses. *Plant Cell* **11**: 273–287
- Salinas-Mondragon RE, Garciduenas-Pina C, Guzman P (1999) Early elicitor induction in members of a novel multigene family coding for highly related RING-H2 proteins in *Arabidopsis thaliana*. *Plant Mol Biol* **40**: 579–590
- Shiu SH, Bleecker AB (2001) Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. *Proc Natl Acad Sci USA* **98**: 10763–10768
- Silverman N, Maniatis T (2001) NF- $\kappa$ B signaling pathways in mammalian and insect innate immunity. *Genes Dev* **15**: 2321–2342
- Takai R, Matsuda N, Nakano A, Hasegawa K, Akimoto C, Shibuya N, Minami E (2002) EL5, a rice N-acetylchitooligosaccharide elicitor-responsive RING-H2 finger protein, is a ubiquitin ligase which functions in vitro in co-operation with an elicitor-responsive ubiquitin-conjugating enzyme, OsUBC5b. *Plant J* **30**: 447–455
- Tao Y, Xie Z, Chen W, Glazebrook J, Chang H-S, Han B, Zhu T, Zou G, Katagiri F (2003) Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *Plant Cell* **15**: 317–330
- Ulevitch RJ, Tobias PS (1999) Recognition of gram-negative bacteria and endotoxin by the innate immune system. *Curr Opin Immunol* **11**: 19–22
- Van den Ackerveken GF, Van Kan JA, De Wit PJGM (1992) Molecular analysis of the avirulence gene *avr9* of the fungal tomato pathogen *Cladosporium fulvum* fully supports the gene-for-gene hypothesis. *Plant J* **2**: 359–366
- Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX, Kono Kurata N, Yano M, Iwata N, Sasaki T (1998) Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *Proc Natl Acad Sci USA* **95**: 1663–1668
- Zhu T, Wang X (2000) Large-scale profiling of the Arabidopsis transcriptome. *Plant Physiol* **124**: 1472–1476
- Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JD, Felix G, Boller T (2004) Bacterial disease resistance in Arabidopsis through flagellin perception. *Nature* **428**: 764–767
- Zwiesler-Vollick J, Plovianich-Jones AE, Nomura K, Bandyopadhyay S, Joardar V, Kunkel BN, He SY (2002) Identification of novel hrp-regulated genes through functional genomic analysis of the *Pseudomonas syringae* pv. *tomato* DC3000 genome. *Mol Microbiol* **45**: 1207–1218