The *Arabidopsis thaliana* Transcriptome in Response to *Agrobacterium tumefaciens*

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The pathogen *Agrobacterium tumefaciens* infects a broad range of plants, introducing the T-DNA into their genome. Contrary to all known bacterial phytopathogens, *A. tumefaciens* lacks the hypersensitive response-inducing *hrp* genes, although it introduces numerous proteins into the plant cell through a type IV secretion system. To understand the timing and extent of the plant transcriptional response to this unusual pathogen, we used an *Arabidopsis* 26,000-gene oligonucleotide microarray. We inoculated *Arabidopsis* cell cultures with an oncogenic *Agrobacterium* strain and analyzed four biological replicates to identify two robust sets of regulated genes, one induced and the other suppressed. In both cases, the response was distinct at 48 h after infection, but not at 24 h or earlier. The induced set includes genes encoding known defense proteins, and the repressed set is enriched with genes characteristic of cell proliferation even though a growth arrest was not visible in the inoculated cultures. The analysis of the repressed genes revealed that the conserved upstream regulatory elements Frankiebox (also known as “site II”) and Telobox are associated with the suppression of gene expression. The regulated gene sets should be useful in dissecting the signaling pathways in this plant–pathogen interaction.

Plants have evolved efficient mechanisms to respond to microorganisms that invade their tissues. Most of these organisms are classified as pathogens and their interaction with plants leads to a set of common defense responses (Hammond-Kosack and Jones 1996; Nimchuk et al. 2003). For example, plants undergo a necrotizing hypersensitive response (HR) to many pathogens, regardless of their nature (Alfano and Collmer 2004). *Agrobacterium tumefaciens* is a soil bacterium that can infect a broad range of plants, introducing a piece of its tumor-inducing (Ti) plasmid, the T-DNA, into the plant genome, the most extreme case of horizontal gene transfer currently known (Brenic and Winans 2005; Gelvin 2003; Nester 2000; Zhu et al. 2000). T-DNA gene expression leads to the synthesis of plant growth regulators, resulting in uncontrolled plant cell division and the crown gall disease (Escobar and Dandekar 2003). Therefore, this bacterium genetically transforms its natural plant host, representing both an intriguing and unique pathogen and a vehicle for genetic engineering. Genetic transformation of its

host is not the only unusual feature of *Agrobacterium tumefaciens*. Contrary to most known bacterial phytopathogens, *A. tumefaciens* lacks the HR-inducing *hrp* genes and does not induce the HR, even if it introduces numerous proteins into the plant cell (Pu and Goodman 1992; Wood et al. 2001). In addition, *A. tumefaciens* is the only phytopathogen that uses a type IV secretion system (Christie and Vogel 2000). These features suggest that the interaction between the host plants and this pathogen has evolved along very different lines from those of typical plant–pathogens interactions.

We know a considerable amount about bacterial genes that is important during plant pathogenesis. The processing and transfer of the T-DNA have been studied intensively and are mediated by a number of Ti plasmid-encoded virulence (Vir) proteins, some of which are transferred to the plant, in addition to the T-DNA (Schrammeijer et al. 2003; Vergunst et al. 2000). In contrast, host responses to this bacterium have not been studied extensively, although this understanding is critical for both practical applications in biotechnology and insights into host–microbe interactions.

Recent studies have begun to reveal how *A. tumefaciens* exploits host factors in order to achieve transformation (Gelvin 2000; Tzfira et al. 2002). Knowledge of these host factors has greatly increased through the identification of Vir protein interactors (Bako et al. 2003; Ballas and Citovsky 1997; Deng et al. 1998; Lacroix et al. 2005; Schrammeijer et al. 2001; Tao et al. 2004; Tzfira et al. 2001, 2004) and genetic screens (Nam et al. 1999; Roberts et al. 2003; Zhu et al. 2003). Although the knowledge of plant-inducing changes in bacterial gene expression has been crucial to our understanding of this interaction, much less is known about overall changes in gene expression in the host. Specifically, we know very little about the timing and type of responses plants mount to *A. tumefaciens* and how this compares with responses to other pathogens, symbionts, and general stresses. Furthermore, little is known about the host response in relation to the transformation process.

Recently, subtractive hybridization and macroarrays were used in combination to identify a number of tobacco genes related to plant defense and cell division or growth that were differentially expressed during *A. tumefaciens* transformation (Veena et al. 2003). Based on these data, the authors suggested a model in which tobacco undergoes a response in the first 6 h, which results in the activation of defense-related genes. The model further proposed that, after 24 h, transfer of T-DNA and vir proteins results in induction of growth-related genes and suppression of defense-related genes. Suppression of a host defense response is an intriguing possibility that would help explain how *A. tumefaciens* sustains a long-term association with plant tissues.

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*The e-Xtra logo stands for “electronic extra” and indicates that Figures 2 through 4 appear in color online.

Our previous studies have used the cDNA-amplified fragment length polymorphism (AFLP) technique to identify changes in gene expression in cell cultures of the plant *Ageratum conyzoides* upon infection with *Agrobacterium tumefaciens* (Ditt et al. 2001). Several of the genes identified are related to plant defense responses and we have shown that many of these genes also are regulated in response to a general stress caused by inoculation with a nonpathogen (*Escherichia coli*). Furthermore, comparison of plant cell wall attachment-proficient (wild-type) and attachment-deficient strains suggested that the first was capable of damping the plant defense response (Ditt et al. 2005). Previous investigations have been hampered by the use of organisms that are good hosts but have limited (tobacco) or no (*Ageratum* spp.) genomic resources.

Several unanswered problems remain in the analysis of plant responses to *A. tumefaciens*: these include the timing, extent, mode of regulation, and specificity of gene expression changes. Here, a microarray system that covers most described genes of the model plant *Arabidopsis* was used to expand our initial analysis. We inoculated *Arabidopsis* cell cultures with an oncogenic *Agrobacterium* strain to examine the timing and extent of regulatory changes, using efficient transformation procedures. We analyzed multiple biological replicates and identified two robust sets of regulated genes, one induced and the other suppressed. The analysis of these genes reveals upstream regulatory elements in *Arabidopsis* which are associated with the suppression of gene expression.

RESULTS

Transformation of an *Arabidopsis thaliana* cell culture line and microarray experimental design.

To expand our expression studies by using microarrays, we established an *Agrobacterium* transient transformation protocol in *Arabidopsis*. Strain A348(pBISN1) (discussed below) contained a construct consisting of a β -glucuronidase (GUS) gene with an artificial intron that was used to monitor transformation efficiency in each experiment. Although transient transformation of *Arabidopsis* organs in the whole plant was inefficient and inconsistent (Ditt et al. 2001, 2005), we obtained improved efficiency with a cell culture line of the *Arabidopsis* Ler ecotype

(May and Leaver 1993) using wild-type strain A348. In addition, a collection of mutants in the A348 background is available and could be used for later comparisons of induced plant responses. Considerable variation was observed in the transformation response; therefore, only experiments resulting in efficient transformation of plant cells (discussed below) were used for microarray analysis. For sample comparison in the microarrays, we chose a reference type of design (Kerr 2003), in which every RNA sample of interest is compared with one single reference RNA. The reference RNA was obtained by pooling RNA extracted from a large number of samples of *Arabidopsis* cells treated like 24-h mock-inoculated controls.

Microarray analysis reveals differential expression in *Arabidopsis* cells at 48 h after *Agrobacterium* sp. inoculation.

In order to identify statistically significant and reproducible changes in gene expression over the time-course of *Agrobacterium* sp. infection, we assayed four independently obtained RNAs from mock-inoculated and *Agrobacterium*-inoculated samples using microarrays (Fig. 1). *Arabidopsis* cell cultures were mock-inoculated (control, discussed below) or inoculated with strain A348. Samples were collected at 4, 12, 24, and 48 h after inoculation and RNA was extracted from the cells. Four separate infection experiments (biological replicates) were carried out at different times. After microarray hybridization and data analysis (discussed below), lists of significant genes were identified using the significance analysis of microarray (SAM) methodology (discussed below). Surprisingly, considering the large number of genes being screened, the first three timepoints yielded no significant changes. For the last timepoint (48 h), strong evidence of differential expression was seen. Results for the 48-h timepoint analysis, including the SAM-estimated "false discovery rate" (FDR) for each list, are shown in Table 1. We focused on a list with the 303 most significant genes for further analysis. The estimated FDR for this set is only 0.5%, (i.e., fewer than two genes are expected to be false positives). Comparison of any two biological replicates with the total of four revealed that a different set of genes would have been identified if only two biological replicates were assigned (data not shown). Remarkably, comparing four paired

Fig. 1. Experimental design for microarray analysis.

biological replicates identified candidate regulated genes that displayed changes in expression well below the commonly used 2× threshold of regulation and eliminated many genes whose stochastic response would reduce reproducibility.

Identity of up- and downregulated genes at 48 h after *Agrobacterium* sp. inoculation.

From the list of 303 unique differentially regulated genes, 115 were upregulated (Table 2) and 188 were downregulated (Table 3) at 48 h after inoculation with *A. tumefaciens*. The regulated genes were grouped into classes using the Gene Ontology (GO) classification adopted by TAIR (discussed below). In each of the up- and downregulated categories, genes belonging to the same classes appear multiple times. For example, among defense- or stress-related genes, four peroxidases, four glutathione transferases or peroxidases, and four enzymes related to secondary metabolism (two caffeoyl-CoA methyltransferases and two cytochrome P450 monooxygenases) were upregulated. Among downregulated genes, a large number of genes encoding ribosomal proteins ($n = 53$) were repressed. Also, among downregulated genes, four actins and two tubulins were identified. Several transcription factors, both up- and downregulated, were differentially expressed. Three transcription factors belonging to the Myb family, which is known to be induced by several stresses (Denekamp and Smeekens 2003), were upregulated by *A. tumefaciens*. Interestingly, one Myb transcription factor (At1g13300) was downregulated in our studies. We also identified two upregulated basic helix-loop-helix (bHLH) transcription factors. Recently, this class of transcription factors has been connected to stress responses in plants (Smolen et al. 2002; Wang et al. 2003). We also identified other stress-related transcription factors, such as a bZIP (induced) and an AP2 domain (repressed) (Chen et al. 2003; Singh et al. 2002), whereas another commonly regulated transcription factor in defense responses, the WRKY (Kalde et al. 2003), was not differentially regulated. Interestingly, a homeodomain transcription factor, *WUSCHEL*, which is involved in meristem regulation (Schoof et al. 2000), was upregulated.

Differential expression in microarrays is confirmed by quantitative RT-PCR.

The differential expression of nine genes selected from Tables 2 and 3 were validated by quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) (discussed below). We selected both known and unknown genes showing different ranges of expression in the microarray analysis. The array measurements of all nine genes were qualitatively confirmed by this analysis. The results for three representative genes are shown in Figure 2. These three genes encompass the two most extreme values and one intermediate value obtained in the RT-PCR analysis. The fold change values obtained in the microarray also are presented for each of these genes, showing the high correlation between microarray and real-time RT-PCR results. The pattern of expression of these genes in the microarray assays during the time course is shown in Figure 2. The three genes encode a lysine and histidine specific transporter (At5g40780), a cell wall invertase (At3g13790) (both upregulated), and a xyloglucan endotransglycosylase (At2g06850) (downregulated). Interestingly, a second lysine and histidine transporter (At1g48640) also was upregulated in the microarray analysis (Table 2). A carrot β -fructosidase/cell wall invertase was reported to be induced by wounding and infection with the bacterial pathogen *Erwinia carotovora* (Sturm and Chrispeels 1990), and gene At3g13790 was induced by bacterial (*Pseudomonas syringae*) and fungal (*Alternaria brassicicola*) inoculation, although it was not induced by wounding (Table 4). Xyloglucan endotransglycosylase is also a cell wall enzyme and,

interestingly, we identified two genes encoding this enzyme in our microarray, one analyzed by RT-PCR (At2g06850), which was downregulated (Table 3), and another (At4g30270), which was upregulated (Table 2). The contrasting regulation of these two related genes in response to *A. tumefaciens* also was observed in response to *Pseudomonas* inoculation and senescence (Table 4). These cell wall enzymes form a large family in *Arabidopsis* and different members can have different responses to the same stimulus (Yokoyama and Nishitani 2001).

Classification of 303 differentially expressed genes into functional categories.

We used the GO annotations tool in the *Arabidopsis thaliana* database to classify genes that were up- and downregulated into functional categories. Results of this classification for the organizational level of biological process are shown in Figure 3. We also recorded, for comparison, the amount expected by chance for each category. For upregulated genes, “response to abiotic or biotic stimulus,” “response to stress,” “electron transport or energy pathways,” “transport,” and “protein metabolism” deviated significantly from the null hypothesis ($P = 0, 0, 0.004, 0.19$, and 0.031 , respectively). For downregulated genes, “cell organization and biogenesis,” “protein metabolism,” and “DNA or RNA metabolism” deviated significantly from the null hypothesis ($P = 0, 0$, and 0.009 , respectively). Thus, the downregulated set of genes is functionally distinct from the upregulated set.

A comparison with other *Arabidopsis* microarray data sets reveals similarities and differences between different treatments.

In our previous studies, we compared *A. tumefaciens*- and *Escherichia coli*-treated plant cells and found that several of the genes altered by *A. tumefaciens* inoculation are part of a general response to stress (Ditt et al. 2001). To gain further insight into this aspect of commonalities and differences between different treatments and to explore the vast amount of information available from *Arabidopsis* microarray data, we compared our results with available data sets from the following seven treatments: inoculation with *Cucumber mosaic virus* (CMV) (Marathe et al. 2004), inoculation with the bacterial pathogen *P. syringae* (Tao et al. 2003), inoculation with the fungal pathogen *Alternaria brassicicola* (van Wees et al. 2003), treatment with a wound-inducing compound (Guan and Nothnagel 2004), senescence-inducing conditions (Lin and Wu 2004), and treatment with the auxin hormones indole-3 acetic acid (IAA) (Redman et al. 2004) and 2,4-dichlorophenoxyacetic acid (2,4-D) (Raghavan et al. 2005) (Table 4). All seven studies used either Affymetrix (Santa Clara, CA, U.S.A.) whole-genome arrays or an Affymetrix array representing 8,000 *Arabidopsis* genes (for *Pseudomonas* and *Alternaria* spp. treatments). From the 303 genes significantly altered by *A. tumefaciens* in our study, only 83 genes overlapped

Table 1. Summary of Significance analysis of microarrays for the 48-h timepoint

Length of gene list	Estimated no. of false calls ^a	Estimated FDR (%) ^b
882	21.0	1.4
551	6.5	0.7
303	2.5	0.5
195	1.5	0.5
130	0.0	0.0

^a Estimated number of false calls under global null.

^b Estimated false discovery rate (FDR). Lists of differentially expressed genes were generated by applying statistical criteria of varying stringency. A more stringent criterion results in a shorter gene list and a small expected number of false discoveries. Similarly, a less stringent criterion gives longer gene lists at the cost of a higher rate of false discoveries.

with at least one of these seven other conditions (Table 4). The largest overlap (53 genes) was with *Pseudomonas* spp.-induced changes. However, this set may have been overestimated because there was no biological replication and the *Pseudomonas* spp.-regulated gene candidates were selected on the basis of a fold change cut-off only. A statistically significant overlap, however, was found with the *Pseudomonas*-, *Alternaria*-, senescence-, and wounding-regulated genes (Table 4). No relationship was observed between *Agrobacterium* sp. treatment and auxin treatments. Therefore, our microarray study indicates that, although we used a wild-type *Agrobacterium* strain, no auxin responses were triggered in the first 48 h by the expression of

genes on the T-DNA. This is consistent with the lack of substantial GUS expression in *Arabidopsis* cells observed at 48 h after inoculation (discussed below), suggesting that there was insufficient time for expression of the hormonal biosynthesis genes located on the T-DNA.

Identification of candidate transcriptional motifs associated with the *Agrobacterium* sp.-regulated gene sets.

We examined the putative promoters of the regulated genes for the presence of possible transcriptional motifs that could contribute to the observed response to *A. tumefaciens*. The pro-

Table 2. *Arabidopsis* genes upregulated 48 h after inoculation with *Agrobacterium tumefaciens*

Locus	Fold change	Description
At4g34050	6.87	Caffeoyl-CoA 3- <i>O</i> -methyltransferase
At2g30490	6.64	Cytochrome P450 73 / trans-cinnamate 4-monooxygenase / cinnamate-4-hydroxylase (CYP73)
At5g36925	5.03	Unknown protein
At5g64120	4.16	<i>Arabidopsis thaliana</i> mRNA for peroxidase ATP15a
At1g22410	4.15	Putative 2-dehydro-3-deoxyphosphoheptonate aldolase (DAHP synthetase)
At5g24760	3.97	Putative alcohol dehydrogenase (ADH)
At2g43120	3.83	Pirin-related protein
At5g36920	3.70	Unknown protein
At1g14870	3.62	Expressed protein, similar to PGPS/D12 (<i>Petunia</i> × <i>hybrida</i>)
At3g03780	3.38	Methionine synthase-related
At5g40780	3.24	Putative lysine and histidine specific transporter
At1g48850	3.18	Putative chorismate synthase (5-enolpyruvylshikimate-3-phosphate phospholyase)
At5g39580	3.15 ^a	Putative peroxidase
At4g15610	2.88	Expressed integral membrane protein common family
At4g26220	2.80	Putative caffeoyl-CoA 3- <i>O</i> -methyltransferase
At3g13790	2.77	Glycosyl hydrolase family 32, β-fructosidase (BFRUCT1), cell wall invertase
At3g01290	2.64	Band 7 family protein similar to hypersensitive-induced response protein (<i>Zea mays</i>)
At1g78380	2.55	Putative glutathione transferase
At1g02920	2.51	Putative glutathione transferase
At2g28340	2.36	GATA zinc finger protein
At1g32100	2.35	Putative pinorensin-lariciresinol reductase
At3g21240	2.26	4-coumarate:CoA ligase 2 (4-coumaroyl-CoA synthase 2) (4CL2)
At2g44370	2.20	DC1 domain-containing protein
At4g08410	2.15	Proline-rich extensin-like family
At4g30270	2.11	Xyloglucan endotransglycosylase (meri5B)
At2g43510	2.10	Trypsin inhibitor-related
At3g54580	2.07	Proline-rich protein family
At3g58890	2.05	Syntaxin-related family protein; contains a novel domain similar to F-box
At3g03960	2.04	Putative chaperonin
At5g13420	2.03	Transaldolase - like protein
At1g52200	2.02	Expressed protein, similar to PGPS/D12 (<i>Petunia</i> × <i>hybrida</i>)
At3g29970	2.00	Germination protein-related
At3g44780	1.98	Unknown protein
At1g78860	1.95	Curculin-like (mannose-binding) lectin family
At5g19440	1.91	Putative cinnamyl-alcohol dehydrogenase (CAD)
At5g42350	1.91	Kelch repeat containing F-box protein family
At2g22880	1.91	VQ motif-containing protein
At1g61660	1.88	Basic helix-loop-helix (bHLH) family protein
At3g43190	1.83	Putative sucrose synthase (UDP-glucose-fructose glucosyltransferase)
At1g51810	1.77	Putative leucine rich repeat protein kinase
At2g36380	1.77	ABC transporter family protein
At5g20960	1.76	Aldehyde oxidase 1 (AO1)
At4g18670	1.75	Leucine-rich repeat extensin family
At3g54590	1.75	Proline-rich protein family
At1g60730	1.74	Aldo/keto reductase family
At4g30190	1.73	Putative ATPase 2, plasma membrane-type (proton pump 2) (proton-exporting ATPase)
At5g06320	1.71	Harpin-induced family protein /NDR1/HIN1-like protein 3 similar to harpin-induced protein hin1
At4g33870	1.71	Putative peroxidase
At3g46270	1.70	Receptor protein kinase-related. Weak similarity to light repressible receptor protein kinase
At1g48640	1.69	Putative lysine and histidine specific transporter
At1g11820	1.67	Glycosyl hydrolase family 17 protein, similar to elicitor inducible chitinase (<i>Nicotiana tabacum</i>)
At2g42140	1.66	VQ motif-containing protein
At1g69850	1.64	Nitrate transporter (NLT1)
At5g01210	1.63	Transferase family
At4g11650	1.63	Osmotin-like protein (OSM34)
At3g44320	1.63	Nitrilase 3
At3g50590	1.62	Transducin / WD-40 repeat protein family

(continued on next page)

^a This fold change value was obtained from the log ratio average of two repeated spots on the array.

motors were defined operationally as the 5' region upstream of the putative start codon, or upstream of transcription starts when known. We used the sequence alignment tools AlignAce and MEME (Bailey and Elkan 1995; Hughes et al. 2000) to identify motifs shared by coregulated sets of genes. To establish the relevance of these motifs to the response, we compared the sequence of motif candidates with the sequence of nucleotide hexamers that were enriched in the promoters of our regulated genes in comparison with the control, which consisted of the total genomic set of promoters. For most motifs upstream of upregulated genes, we could not gather sufficient evidence for significance. In the set of repressed genes, however, we found two motifs whose analysis indicated a high probability for their

association with downregulated genes (Fig. 4 and Table 5). One motif we called Frankiebox is defined by the sequence WWTGGGCTWW (W=A or T; Y=C or T). This motif occurred in 47 of the 188 suppressed genes. Of great significance is the fact that Frankiebox corresponds to the "motif or site II" identified in the promoter of the gene encoding the Proliferating Cell Nuclear Antigen (Kosugi et al. 1991, 1995). Given the generality of the name "motif II", we decided to keep the name Frankiebox. The other motif is defined by the sequence TAGGKTTT (K=G or T) (Fig. 4). This sequence corresponds to the Telobox, first identified in the promoter of the gene encoding the elongation factor 1 and also present in the promoter of ribosomal protein genes (Axelos et al. 1989; Regad et al.

Table 2. (continued from preceding page)

Locus	Fold change	Description
At4g38540	1.62	Monooxygenase 2 (MO2)
At2g16720	1.61	Myb DNA-binding protein
At2g21490	1.61	Dehydrin protein family
At1g65295	1.60	Unknown protein
At2g26560	1.59	Putative patatin
At1g79180	1.58	Myb family transcription factor
At1g78780	1.56	Pathogenesis-related family protein (<i>Hordeum vulgare</i>)
At4g38980	1.55	Unknown protein
At5g38900	1.55	DSBA oxidoreductase family protein
At3g01570	1.53	Glycine-rich protein/oleosin
At1g34510	1.52	Putative peroxidase
At1g44940	1.51	Sequence of BAC T12C22 from <i>Arabidopsis thaliana</i> chromosome 1
At2g22890	1.51	Unknown protein
At2g17220	1.50	Protein kinase -related
At1g56250	1.50	F-box protein (SKP1 interacting partner 3-related)
At5g12970	1.50	C2 domain-containing protein
At5g65110	1.50	Acyl-CoA oxidase
At5g62070	1.48	Calmodulin-binding family protein
At5g59616	1.48	Protein kinase-related; contains similarity to light repressible receptor protein kinase
At2g22850	1.47	bZIP family transcription factor
At2g31570	1.46	Putative glutathione peroxidase
At5g59230	1.44	Transcription factor-related. Low similarity to transcription factor IIA large subunit
At4g30210	1.43	Putative NADPH-ferrihemoprotein reductase (NADPH-cytochrome p450 reductase)
At4g19360	1.42	Unknown protein
At3g21070	1.42	ATP-NAD kinase family protein
At1g69410	1.41	Eukaryotic initiation factor 5A-related
At1g60680	1.39	Aldo/keto reductase family
At2g01905	1.37	<i>A. thaliana</i> cyclin J18 (cycJ18)
At1g33720	1.37	Cytochrome P450 monooxygenase
At5g18900	1.36	Oxidoreductase, 2OG-Fe(II) oxygenase family
At1g06310	1.36	Acyl-CoA oxidase ACX3 -related
At3g17810	1.36	Dehydrogenase -related
At3g48000	1.36	Putative mitochondrial aldehyde dehydrogenase (ALDH)
At3g58740	1.35	Citrate synthase -related protein
At5g20230	1.35	Plastocyanin-like domain containing protein
At3g51430	1.34	Strictosidine synthase-related
At2g17950	1.34	Homeodomain transcription factor (WUSCHEL)
At1g06920	1.34	Ovate family protein
At2g27190	1.34	Iron(III)-zinc(II) purple acid phosphatase (precursor)
At1g69570	1.33	Dof zinc finger protein
At1g62800	1.33	Aspartate aminotransferase, cytoplasmic isozyme 2 (transaminase A/Asp4)
At4g26690	1.32	Glycerophosphoryl diester phosphodiesterase family protein
At1g06030	1.32	Putative fructokinase
At1g13990	1.31	Unknown protein
At4g38220	1.31	Putative aminoacylase/N-acyl-L-amino-acid amidohydrolase
At4g11600	1.30	Putative glutathione peroxidase
At3g61950	1.29	Basic helix-loop-helix (bHLH) family protein
At2g31865	1.28	Poly(ADP-ribose) glycohydrolase -related
At4g01680	1.27	Myb family transcription factor
At4g15390	1.25	Acyltransferase family
At1g02205	1.25	CER1 protein
At1g55450	1.25	Embryo-abundant protein-related
At3g30821	1.25	Gypsy-like retrotransposon family (Athila)
At5g38130	1.23	Hydroxycinnamoyl/benzoyltransferase-related
At2g32820	1.21	Unknown protein
At3g02210	1.20	Phytochelatinsynthetase family protein/COBRA cell expansion protein
At4g02760	1.19	F-box protein family
At2g32250	1.18	Far-red impaired responsive protein

Table 3. *Arabidopsis* genes downregulated 48 h after inoculation with *Agrobacterium tumefaciens*

Locus	Fold change	Description
At3g04110	0.87	Glutamate receptor family (GLR1)
At1g71170	0.87	Dehydrogenase-related
At3g03250	0.85	UDP-glucose pyrophosphorylase-related
At5g57610	0.85	Protein kinase family; similar to protein kinase (<i>Glycine max</i>)
At1g61410	0.85	tolA protein-related
At4g38240	0.84	Putative α -1,3-mannosyl-glycoprotein β -1,2-N-acetylglucosaminyltransferase
At1g05640	0.84	Ankyrin repeat protein family
At5g01620	0.84	Unknown protein
At1g30880	0.84	Unknown protein
At2g45100	0.83	Transcription factor IIIB 70 KD subunit (TFIIIB)-related
At1g06770	0.83	Zinc finger (C3HC4-type RING finger) family protein
At2g47610	0.83	60S ribosomal protein L7A (RPL7aA)
At5g56510	0.83	Putative pumilio-family RNA-binding protein
At1g56070	0.83	Elongation factor -related
At5g26850	0.83	Similar to cyclin-like protein (<i>Oryza sativa</i> [japonica cultivar-group]) e-127 ^a
At2g02480	0.82	Prokaryotic DNA polymerase III gamma subunit-related
At3g46520	0.82	Actin 12
At5g23740	0.82	40S ribosomal protein S11 (RPS11C)
At4g30480	0.82	Tetratricopeptide repeat (TPR)-containing protein
At5g49530	0.81	SIN-like family protein. Low similarity to Sex-lethal interactor (<i>Drosophila melanogaster</i>)
At3g56490	0.81	Protein kinase C inhibitor-related protein
At5g43680	0.81	Unknown protein
At1g53210	0.81	Sodium/calcium exchanger family protein/ calcium-binding EF hand family protein
At2g14160	0.81	RNA recognition motif (RRM)-containing protein
At1g63660	0.81	Putative GMP synthase [glutamine-hydrolyzing] (glutamine amidotransferase)
At2g37500	0.81	Glutamate/ornithine acetyltransferase -related
At5g16390	0.81	Biotin carboxyl carrier protein 1 (BCCP1)
At3g05590	0.81	60S ribosomal protein L18 (RPL18B)
At5g16130	0.81	40S ribosomal protein S7 (RPS7C)
At2g36170	0.80	Ubiquitin extension protein (UBQ2)/60S ribosomal protein L40 (RPL40A)
At1g77670	0.80	Aminotransferase family
At3g61110	0.80	40S ribosomal protein S27 (RPS27B)
At5g67220	0.80	Nitrogen regulation protein family (NIFR3)
At2g37620	0.80 ^b	Actin 3
At3g24020	0.80	Disease resistance response protein-related
At3g02960	0.80	Copper-binding protein-related
At3g62920	0.80	Unknown protein
At2g19730	0.80	60S ribosomal protein L28 (RPL28A)
At3g53750	0.80	Actin (ACT3)
At4g26300	0.79	Arginyl-tRNA synthetase
At4g27600	0.79	pfkB type carbohydrate kinase protein family
At1g76880	0.79	GT-related trihelix DNA-binding protein
At1g06750	0.79	Expressed protein, similar to H1-lflk (<i>Arabidopsis thaliana</i>)
At3g17730	0.79	No apical meristem (NAM) protein family
At4g17950	0.79	DNA-binding family protein
At4g11080	0.79	High mobility group (HMG1/2) family protein
At4g19390	0.79	Unknown protein
At3g15750	0.79	Unknown protein
At2g47110	0.79	Ubiquitin extension protein (UBQ6)/40S ribosomal protein S27A (RPS27aB)
At1g80410	0.79	Acetyltransferase-related
At1g10417	0.79	Unknown protein
At4g12390	0.79	Pectinesterase-related
At5g62300	0.79	40S ribosomal protein S20 (RPS20C)
At3g45030	0.78	40S ribosomal protein S20 (RPS20A)
At3g53730	0.78	Histone H4
At3g15000	0.78	Expressed protein, similar to DAG protein (required for chloroplast differentiation and palisade development) (<i>Antirrhinum majus</i>)
At5g57090	0.78	Auxin transport protein (EIR1)
At5g62700	0.78	Tubulin β -2/ β -3 chain (TUB3)
At4g10450	0.78	60S ribosomal protein L9 (RPL90D)
At1g48830	0.78	40S ribosomal protein S7 (RPS7A)
At1g22090	0.78	Unknown protein
At1g34030	0.78	40S ribosomal protein S18 (RPS18B)
At4g18730	0.78	60S ribosomal protein L11 (RPL11C)
At5g17090	0.78	Unknown protein
At5g35360	0.78	Acetyl-CoA carboxylase
At1g31812	0.77	Acyl-CoA binding protein-related

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^a These similarities were obtained by comparing the *Arabidopsis* sequence used to design the oligo with the complete database using BLASTp at the National Center for Biotechnology Information. The score obtained is shown.

^b This fold change value was obtained from the log ratio average of 17 repeated spots on the array.

Table 3. (continued from preceding page)

Locus	Fold change	Description
At3g22440	0.77	Hydroxyproline-rich glycoprotein family
At5g09500	0.77	40S ribosomal protein S15 (RPS15C)
At2g27330	0.77	RNA recognition motif (RRM)-containing protein
At5g47930	0.77	Ribosomal protein S27
At5g07090	0.77	40S ribosomal protein S4 (RPS4B)
At3g49250	0.77	Unknown protein
At4g15790	0.77	Unknown protein
At3g12030	0.77	Expressed protein, similar to membrane protein (<i>Homo sapiens</i>)
At1g08360	0.77	60S ribosomal protein L10A (RPL10aA)
At2g36200	0.77	Kinesin-related protein
At1g26740	0.77	Expressed protein, similar to 50S ribosomal protein L32 (<i>Thermus thermophilus</i>)
At3g02560	0.77	40S ribosomal protein S7 (RPS7B)
At3g09500	0.77	60S ribosomal protein L35 (RPL35A)
At1g15370	0.76	Similar to putative chloroplastic RNA-binding protein, with alternative splicing isoforms (<i>O. sativa</i> [<i>japonica</i> cultivar-group]) 4e-68 ^a
At3g12060	0.76	Similar to lustrin A-like (<i>Oryza sativa</i> [<i>japonica</i> cultivar-group]) e-150 ^a
At1g34210	0.76	Somatic embryogenesis receptor-related kinase
At3g25140	0.76	Glycosyltransferase family 8
At5g65010	0.76	Asparagine synthetase
At2g15400	0.76	DNA-directed RNA polymerase II, third largest subunit
At2g42740	0.76	60S ribosomal protein L11 (RPL11A)
At2g20880	0.76	AP2 domain transcription factor
At1g79870	0.76	Oxidoreductase family
At2g33370	0.76	60S ribosomal protein L23 (RPL23B)
At1g43170	0.76	60S ribosomal protein L3 (RPL3A)
At1g34430	0.76	Dihydrolipoamide S-acetyltransferase -related
At3g19080	0.76	SWIB complex BAF60b domain-containing protein
At1g26480	0.76	14-3-3 protein GF14 iota (grf12)
At1g15420	0.75	Unknown protein
At3g46560	0.75	Small zinc finger-related protein TIM9
At4g28440	0.75	DNA-binding protein-related. Contains weak similarity to G-quartet DNA binding protein 3 (<i>Tetrahymena thermophila</i>)
At4g36130	0.75	60S ribosomal protein L8 (RPL8C)
At1g72370	0.75	40S ribosomal protein SA (RPSaA)
At1g04270	0.75	40S ribosomal protein S15 (RPS15A)
At5g12840	0.75	CCAAT box binding factor/ transcription factor Hap2a
At3g55630	0.75	Folylpolyglutamate-dihydrofolate synthase (dhfs/fpgs4)
At2g22870	0.75	Similar to putative nucleotide-binding protein (<i>Arabidopsis thaliana</i>) e-125 ^a
At5g13630	0.74	Putative chloroplast magnesium-chelatase subunit (CHLH)
At1g31350	0.74	F-box family protein
At5g10360	0.74	40S ribosomal protein S6 (RPS6B)
At3g62250	0.74	Ubiquitin extension protein (UBQ5)/40S ribosomal protein S27A (RPS27aC)
At3g25600	0.74	Putative calmodulin
At4g29360	0.74	Glycosyl hydrolase family 17 protein, similar to elicitor inducible chitinase (<i>Nicotiana tabacum</i>)
At3g54260	0.74	Unknown protein
At5g27770	0.74	60S ribosomal protein L22 (RPL22C)
At1g35780	0.73	Unknown protein
At3g56340	0.73	40S ribosomal protein S26 homolog
At5g47530	0.73	Putative auxin-induced protein
At3g43600	0.73	Aldehyde oxidase 2 (AO2)
At2g05220	0.73	40S ribosomal protein S17 (RPS17B)
At5g13850	0.73	Nascent polypeptide-associated complex (NAC) domain-containing protein similar to α -NAC, nonmuscle form (<i>Mus musculus</i>)
At1g51060	0.73	Putative histone H2A
At1g36240	0.73	60S ribosomal protein L30 (RPL30A)
At1g18540	0.73	60S ribosomal protein L6 (RPL6A)
At4g07960	0.73	Glycosyltransferase family 2
At1g50010	0.72	Tubulin α -2/ α -4 chain (TUA2)
At4g03170	0.72	Similar to DNA-binding protein-related (<i>A. thaliana</i>) 8e-87 ^a
At5g61170	0.72	40S ribosomal protein S19 (RPS19C)
At5g09810	0.72	Actin 2/7
At5g41685	0.72	Mitochondrial import receptor subunit TOM7 / translocase of outer membrane 7 kDa subunit (TOM7.1)
At2g37190	0.72	60S ribosomal protein L12 (RPL12A)
At2g23930	0.72	Small nuclear ribonucleo protein E -related
At2g20890	0.72	Similar to chloroplast thylakoid formation 1 (<i>A. thaliana</i>) e-163 ^a
At3g27080	0.71	Mitochondrial import receptor subunit TOM20-3/ translocase of outer membrane 20 kDa subunit 3 (TOM20-3)
At4g00430	0.71	<i>A. thaliana</i> mRNA for transmembrane protein
At2g21860	0.71	Violaxanthin de-epoxidase-related
At3g53940	0.71	Mitochondrial carrier protein family
At1g20580	0.71	Putative small nuclear ribonucleoprotein/ putative similar to small nuclear ribonucleoprotein Sm D3 (snRNP core protein D3, Sm-D3) (<i>M. musculus</i>)
At1g13380	0.71	Similar to putative fiber protein Fb34 (<i>O. sativa</i> [<i>japonica</i> cultivar-group]) 2e-61 ^a
At2g29440	0.70	Putative glutathione transferase

(continued on next page)

Table 3. (continued from preceding page)

Locus	Fold change	Description
At5g61130	0.70	Glycosyl hydrolase family protein 17, similar to β -1,3-glucanase (<i>Camellia sinensis</i>)
At4g33865	0.70	40S ribosomal protein S29 (RPS29C)
At3g44010	0.70	40S ribosomal protein S29 (RPS29B)
At4g13170	0.70	60S ribosomal protein L13A (RPL13aC)
At1g41830	0.70	Multi-copper oxidase type I family protein similar to pollen-specific BP10 protein (<i>Brassica napus</i>)
At3g11250	0.69	60S acidic ribosomal protein P0 (RPP0C)
At4g24960	0.69	Abscisis acid-induced-like protein
At5g46430	0.69	60S ribosomal protein L32 (RPL32B)
At5g45775	0.69	60S ribosomal protein L11 (RPL11D)
At5g42060	0.69	Unknown protein
At5g02960	0.69	40S ribosomal protein S23 (RPS23B)
At3g04400	0.68	60S ribosomal protein L23 (RPL23C)
At5g64140	0.68	40S ribosomal protein S28 (RPS28C)
At2g16600	0.68	Cytosolic cyclophilin (ROC3)
At5g59850	0.68	40S ribosomal protein S15A (RPS15aF)
At4g02800	0.68	Unknown protein
At4g14320	0.68	60S ribosomal protein L36a/L44 (RPL36aB)
At5g28060	0.67	40S ribosomal protein S24 (RPS24B)
At3g07590	0.67	Small nuclear ribonucleo protein (Sm-D1) -related
At3g23390	0.67	60S ribosomal protein L36a/L44 (RPL36aA)
At4g23000	0.67	Calcineurin-like phosphoesterase family
At2g42570	0.67	Similar to lustrin A-like (<i>O. sativa</i> [japonica cultivar-group]) e-116 ^a
At4g34870	0.66	Peptidylprolyl isomerase (cyclophilin)
At4g15802	0.66	Unknown protein
At5g45300	0.66	Glycosyl hydrolase family 14 (β -amylase)
At1g60030	0.66	Permease -related
At2g27970	0.65	CDK-subunit 2
At4g30330	0.65	Small nuclear ribonucleo protein homolog
At4g38660	0.65	Putative thaumatin-like protein
At5g48300	0.65	Glucose-1-phosphate adenylyltransferase, small subunit, chloroplast (ADP-glucose pyrophosphorylase) (APS1)
At1g24280	0.65	Glucose-6-phosphate 1-dehydrogenase
At5g59870	0.65	Putative histone H2A
At2g25210	0.65	60S ribosomal protein L39 (RPL39A)
At3g16780	0.65	60S ribosomal protein L19 (RPL19B)
At2g16830	0.64	Unknown protein
At2g44830	0.64	Putative protein kinase
At3g59540	0.64	60S ribosomal protein L38 (RPL38B)
At5g27120	0.63	Putative SAR DNA-binding protein
At3g21190	0.62	Unknown protein
At2g23120	0.62	Unknown protein
At1g22880	0.62	Glycosyl hydrolase family 9
At1g41880	0.61	60S ribosomal protein L35a (RPL35aB)
At5g56670	0.61	40S ribosomal protein S30 (RPS30C)
At2g46890	0.60	Unknown protein
At3g21770	0.60	Putative peroxidase
At1g13300	0.58	Myb family transcription factor
At3g14310	0.57	Pectin methylesterase -related
At5g01600	0.57	Ferritin 1 precursor
At3g16240	0.57	Delta tonoplast integral protein (delta-TIP)
At4g08685	0.56	Pollen Ole e 1 allergen and extensin family protein
At3g08030	0.51	Unknown protein
At2g02100	0.50	Putative plant defensin protein (PDF2.2)
At2g06850	0.24	Xyloglucan endotransglycosylase (ext/EXGT-A1)

1994; Tremousaygue et al. 2003). The occurrence of the Frankiebox and its joint occurrence with the Telobox were highly significant. The relative position of these motifs in the upstream region was conserved, because the Frankiebox occurred one to five times in each promoter and frequently as pairs 10 to 50 bp apart. The Telobox, typically, was proximally located with respect to the Frankiebox. Comparison of the motifs across the downregulated gene set indicated that they were positioned preferentially around nucleotide -80 from the start codon (Fig. 5) and predominantly in the 500-bp region proximal to the regulated genes (Table 6). In all, 27 of 49 genes encoding the downregulated ribosomal proteins carried the motif. The frequency and position of these motifs make them good candidates for regulatory elements involved in transcriptional suppression of genes in response to *A. tumefaciens*.

DISCUSSION

In plant-pathogen interactions, a number of microarray expression studies have identified similarities and distinctions between different defense pathways (Schenk et al. 2000, 2003; Tao et al. 2003; Wan et al. 2002) and also have revealed regulatory elements in co-regulated genes (Maleck et al. 2000). A comprehensive analysis using microarrays, in particular an analysis of the timing of plant responses, had not been done before for the *Arabidopsis*-*A. tumefaciens* interactions. Here, we used an oligonucleotide array representing virtually the complete predicted gene set of *Arabidopsis* to identify genes regulated by infection with wild-type *A. tumefaciens*. Although a high level of biological replication is becoming a standard in many systems, it is uncommon in plant-microarray studies. Biological replication, combined

with sound statistical analysis, allowed us to identify genes that are truly differentially expressed.

We found lack of substantial gene regulation at 24 h and earlier, and this was confirmed by real-time RT-PCR for selected genes of the set regulated at 48 h. It is possible that some genes responded to *A. tumefaciens* infection at earlier timepoints; however, these genes did not achieve high levels of statistical significance and were below the level of detection for our analysis. This delayed regulatory pattern is remarkable by comparison with other plant pathogens interacting with plants and differs from the results of Veena and associates (2003). In that report, candidate genes for a general defense response were activated 6 to 12 h after inoculation. The same genes were found to be suppressed at 24 to 36 h. This study differed from the present one in plant system and methodology. Veena and associates used tobacco cells, different approaches to the identification of differentially expressed genes, and pooled five experiments in one RNA extraction. These and other less-obvious differences may explain the discrepancy in the results obtained.

Consistent with our study with *Ageratum* spp. infection, genes belonging to the same classes were found to be activated in *Arabidopsis* at 48 h; these include genes involved in transcription regulation, cell wall metabolism, defense, secondary metabolism, and carbohydrate metabolism. Based on the level of activation and in order of decreasing significance, the categories of induced genes identified in the present study were response to stress and response to abiotic or biotic stimuli, which included defense-related genes, electron transport or energy pathways, transport, and protein metabolism.

A surprising finding from our microarray studies was the nature of the downregulated set of genes which, in order of decreasing significance, belonged to the classes of cell organization and biogenesis, protein metabolism, and nucleic acid metabolism. For example, a large number of ribosomal protein genes were identified. This response is consistent with a slow-down in proliferative cell metabolism, suggesting a transition to a resting state. Remarkably, there was no evidence at 48 h for a slow-down in growth of the infected cell suspension. Very little information is available about conditions that repress these pathways, especially in plants. One report identified a single ribosomal protein gene being downregulated in soybean by inoculation with bacterial and fungal pathogens and by treatment with heavy metals (Ludwig and Tenhaken 2001). An overall repression of the translation machinery as a result of downregulation of several genes encoding large and small ribosomal proteins was observed in the cyanobacterium *Synechocystis* in response to UV-B light (Huang et al. 2002). In contrast, upregulation of several ribosomal protein genes was reported in maize exposed to UV-B light (Casati and Walbot 2003). Also, in soybean, three ribosomal protein genes were induced by low temperature (Kim et al. 2004). Inhibition of protein synthesis has been observed in mammalian cells during apoptosis (Jeffrey et al. 2002), cancer (Lui et al. 2005), hypoxia caused by high energy-consuming processes (Casey et al. 2002), and even during neuronal differentiation (Bevort and Leffers 2000). These examples indicate that different kinds of stresses or major cell reorganization processes, such as differentiation, can result in drastic changes in the expression pattern of ribosomal protein genes. This, in turn, could lead to the reorganization of the expression of other proteins, in order to adapt to the different conditions. However, it is interesting that this simultaneous effect on such a large number of ribosomal protein genes has not been reported for other plant pathogens. Veena and associates (Veena et al. 2003) also identified several ribosomal protein genes altered by *Agrobacterium* inoculation, but they were upregulated in their study. In addition to ribosomal protein genes, we found other cell division or growth-

related genes downregulated in our studies, such as three histones, four actins, two tubulins, and one cyclin-like protein.

The identification of the Frankiebox and Telobox motifs in the upstream regions of the downregulated gene set indicates that they share a close regulatory relationship. These motifs are strongly associated with these genes and bear stereotypical positions in the putative promoter regions. The same motifs previously have been identified as elements required for a proliferative response by genomic, binding, and transgenic studies (Kosugi and Ohashi 1997; Kosugi et al. 1991, 1995; Manevski et al. 2000; Tremousaygue et al. 1999). In one study, Frankiebox (previously called element II) and Telobox worked synergistically to confer meristem-specific expression of a transgene driven by a minimal promoter (Tremousaygue et al. 2003). Our results confirm the association of these regulatory elements with genes involved in proliferation and are consistent with a role in coordinate repression by *Agrobacterium* infection.

The set of downregulated genes also includes several defense-related genes, although more upregulated genes were observed in this category. These genes include an elicitor-inducible chitinase, a β -1,3-glucanase, a thaumatin-like protein, a peroxidase, and hydroxyproline-rich glycoproteins, among others (Table 3). Particularly interesting is the downregulation of a gene (PDF2.2) which is a member of the plant defensin family protein, a commonly used marker of jasmonate-mediated induced

Fig. 2. Expression comparison of regulated genes. The real-time polymerase chain reaction (PCR) analysis displays the ratio of induced to mock-induced for each gene. The microarray analysis displays the expression change for each biological replicate relative to the standard.

defenses (Brown et al. 2003). These results support the idea that *A. tumefaciens* may suppress plant defense responses (Ditt et al. 2005; Veena et al. 2003). However, the significant upregulation of numerous defense-related genes suggests that induction and repression of specific responses are balanced during *A. tumefaciens*–plant interactions.

The use of a cell culture of *Arabidopsis thaliana* facilitated the present study. This system was validated by the identification of the induced gene At5g40780, which encodes a lysine and histidine transporter. The same gene also was identified in a previous cDNA-AFLP study of *Agrobacterium* infection of *Arabidopsis* root tissue (*unpublished observations*). A second

lysine/histidine transporter (At1g48640) also was upregulated by *A. tumefaciens* in the microarray analysis, suggesting that these transporters may play an important role in the *Agrobacterium* interaction with *Arabidopsis* cells. Another interesting aspect of these amino acid transporters is that, except for IAA, no other treatments with which we compared our data (Table 4) resulted in induction or repression of these genes. This could indicate a specific role in *A. tumefaciens*–plant interactions. In fact, most genes ($n = 220$) from our set of 303 genes differentially regulated by *A. tumefaciens* were not induced by any of the seven other conditions of stress or hormonal treatments with which we compared our results.

Table 4. Comparison of *Agrobacterium* (Agro)-regulated genes at 48 h with available datasets from biotic, abiotic, and hormonal treatments on *Arabidopsis*^a

Agro	Fold change	CMV ^b	Pse ^c	Alt ^d	Woun ^e	Sen ^f	IAA ^g	2,4D ^h	Description
At4g34050	6.87	...	+	+	...	...	...	...	Caffeoyl-CoA 3-O-methyltransferase
At2g30490	6.64	...	+	+	...	...	...	...	Cytochrome P450 73 / trans-cinnamate 4-monooxygenase/cinnamate-4-hydroxylase (CYP73) (C4H)
At5g64120	4.16	...	+	+	–	...	+	...	<i>Arabidopsis thaliana</i> mRNA for peroxidase ATP15a
At5g39580	3.15	...	+	+	–	...	+	...	Putative peroxidase
At1g14870	3.62	...	...	...	+	+	...	...	Expressed protein, similar to PGPS/D12 (<i>Petunia × hybrida</i>)
At5g40780	3.24	...	...	...	...	...	+	...	Putative lysine and histidine specific transporter
At1g48850	3.18	...	...	...	...	–	...	...	Putative chorismate synthase (5-enolpyruvylshikimate-3-phosphate phospholyase)
At4g15610	2.88	...	+	+	...	+	...	...	Expressed integral membrane protein common family
At3g13790	2.77	...	+	+	...	–	+	...	Glycosyl hydrolase family 32, β-fructosidase (BFRUCT1)/β-fructofuranosidase/cell wall invertase
At3g01290	2.64	...	...	...	+	+	...	...	Band 7 family protein similar to hypersensitive-induced response protein (<i>Zea mays</i>)
At1g78380	2.55	...	+	+	...	...	...	...	Putative glutathione transferase
At1g02920	2.51	...	+	+	...	+	...	...	Putative glutathione transferase
At3g21240	2.26	...	...	+	...	...	...	...	4-coumarate:CoA ligase 2 (4-coumaroyl-CoA synthase 2) (4CL2)
At2g44370	2.20	...	+	...	...	...	...	...	DC1 domain-containing protein
At4g30270	2.11	...	+	+	...	+	...	–	Xyloglucan endotransglycosylase (meri5B)
At2g43510	2.10	...	+	+	...	...	...	...	Trypsin inhibitor-related
At1g52200	2.02	...	...	...	+	...	...	...	Expressed protein, similar to PGPS/D12 (<i>Petunia × hybrida</i>)
At1g78860	1.95	...	+	+	+	...	...	...	Curculin-like (mannose-binding) lectin family
At1g61660	1.88	...	+	...	...	...	...	...	Basic helix-loop-helix (bHLH) family protein
At5g20960	1.76	...	+	+	...	...	...	...	Aldehyde oxidase 1 (AO1)
At1g11820	1.67	...	–	...	...	...	...	...	Glycosyl hydrolase family 17 protein, similar to elicitor inducible chitinase (<i>Nicotiana tabacum</i>)
At1g69850	1.64	...	–	...	...	...	...	...	Nitrate transporter (NIT1)
At5g01210	1.63	...	...	+	...	...	...	...	Transferase family
At4g11650	1.63	...	+	+	...	...	...	...	Osmotin-like protein (OSM34)
At3g44320	1.63	...	...	...	...	+	...	...	Nitrilase 3
At4g38540	1.62	...	+	+	...	+	...	...	Monooxygenase 2 (MO2)
At1g78780	1.56	...	+	...	+	...	...	...	Pathogenesis-related family protein. Contains similarity to pathogen-related protein (<i>Hordeum vulgare</i>)
At2g22890	1.51	...	...	...	+	...	...	...	Unknown protein
At2g17220	1.50	...	+	+	...	...	...	...	Protein kinase –related
At5g12970	1.50	...	...	...	–	...	...	...	C2 domain-containing protein
At5g65110	1.50	...	+	...	...	+	...	...	Acyl-CoA oxidase
At2g31570	1.46	...	+	+	...	...	...	...	Putative glutathione peroxidase
At4g30210	1.43	...	+	...	...	...	...	...	Putative NADPH-ferrihemoprotein reductase (NADPH-cytochrome p450 reductase)

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^a All 303 genes from Tables 2 and 3 were compared and a total of 83 genes were found to overlap with at least one of these datasets; + indicates that the direction of the change (up or down) is the same we observed and – indicates that the direction is opposite to what we observed.

^b A set of 444 genes (from a total of approximately 24,000) regulated upon inoculation with *Cucumber mosaic virus* (CMV) for different periods of time was used for this comparison (Marathe et al. 2004).

^c A set of 2,970 genes (from a total of approximately 8,000) regulated upon inoculation with any of a number of different *Pseudomonas syringae* strains for different periods of time was used for this comparison (Tao et al. 2003).

^d A set of 640 genes (from a total of approximately 8,000) regulated upon inoculation with *Alternaria brassicicola* for different periods of time was used for this comparison (van Wees et al. 2003).

^e A set of 651 genes (from a total of approximately 24,000) regulated upon treatment with a Yariv phenylglycoside compound that triggers wound-like responses was used for this comparison (Guan and Nothnagel, 2004).

^f A set of 588 genes (from a total of approximately 24,000) regulated upon dark-induced senescence for different periods of time was used for this comparison (Lin and Wu 2004).

^g A set of 325 genes (from a total of approximately 24,000) regulated upon treatment with different concentrations of indole-3 acetic acid (IAA) was used for this comparison (Redman et al. 2004).

^h A set of 174 genes (from a total of approximately 24,000) regulated upon treatment with 1 mM 2,4-dichlorophenoxyacetic acid (2,4-D) for 1 h was used for this comparison (Raghavan et al. 2005).

In conclusion, our study of *Arabidopsis* infection by *Agrobacterium tumefaciens* has demonstrated a delayed plant response in which some general defense genes are activated and proliferative genes are suppressed. It is likely that the robust set of genes we have described may be the consequence of the action of different regulatory pathways. One pathway most likely is defined by the set of Frankiebox- and Telobox-regulated genes. Other pathways may respond to different components of *A. tumefaciens* (such as cell wall and secreted proteins) and to different stages of its association with plant cells (such as binding and transformation). Future studies will compare microarray expression profiles of plants inoculated with different pathogens and

nonpathogens to identify genes regulated by all pathogens and those uniquely regulated by *A. tumefaciens*. In addition, inoculation with different *Agrobacterium* mutants may reveal the bacterial components responsible for changes in plant gene expression and help elucidate the mechanism of the plant response to this intriguing and unique plant pathogen.

MATERIALS AND METHODS

Cell cultures conditions and treatments.

An *Arabidopsis thaliana* cell culture (May and Leaver 1993) was a gift of C. Lisenbee (Mayo Clinic, Scottsdale, AZ,

Table 4. (continued from preceding page)

Agro	Fold change	CMV ^b	Pse ^c	Alt ^d	Woun ^e	Sen ^f	IAA ^g	2,4D ^h	Description
At3g17810	1.36	...	...	...	...	+	...	...	Dehydrogenase-related
At5g20230	1.35	...	+	+	...	+	...	...	Plastocyanin-like domain containing protein
At2g27190	1.34	...	-	...	...	...	...	...	Iron(III)-zinc(II) purple acid phosphatase (precursor)
At4g26690	1.32	...	-	...	...	...	...	...	Glycerophosphoryl diester phosphodiesterase family protein
At1g13990	1.31	...	+	+	...	+	...	...	Unknown protein
At4g11600	1.30	...	+	...	...	+	...	...	Putative glutathione peroxidase
At3g61950	1.29	...	...	...	-	...	...	...	Basic helix-loop-helix (bHLH) family protein
At2g31865	1.28	...	+	...	...	...	...	...	Poly(ADP-ribose) glycohydrolase -related
At1g02205	1.25	-	-	...	...	...	...	...	CER1 protein
At1g55450	1.25	...	-	+	-	...	...	+	Embryo-abundant protein-related
At2g32250	1.18	...	+	...	...	...	...	...	Far-red impaired responsive protein
At1g30880	0.84	...	-	...	...	...	...	...	Unknown protein
At3g46520	0.82	...	+	...	...	...	...	...	Actin 12
At5g16390	0.81	...	+	...	...	...	...	...	Biotin carboxyl carrier protein 1 (BCCP1)
At3g61110	0.80	...	...	+	...	...	...	...	40S ribosomal protein S27 (RPS27B)
At4g26300	0.79	...	+	...	...	...	...	...	Arginyl-tRNA synthetase
At4g27600	0.79	...	+	...	...	...	...	...	pfkB type carbohydrate kinase protein family
At4g17950	0.79	...	-	...	...	...	...	...	DNA-binding family protein
At4g19390	0.79	...	+	...	...	...	...	...	Unknown protein
At4g12390	0.79	...	+	...	...	...	...	...	Pectinesterase - related
At3g53730	0.78	...	...	...	+	...	...	...	Histone H4
At5g35360	0.78	...	+	...	...	...	...	...	Acetyl-CoA carboxylase
At2g27330	0.77	...	-	...	...	...	...	...	RNA recognition motif (RRM)-containing protein
At1g34210	0.76	...	-	...	...	...	...	...	Somatic embryogenesis receptor-related kinase
At2g20880	0.76	+	-	...	...	...	...	...	AP2 domain transcription factor
At5g13630	0.74	...	+	...	...	+	...	...	Putative chloroplast magnesium-chelatase subunit (CHLH)
At3g54260	0.74	...	...	...	+	...	...	...	Unknown protein
At1g51060	0.73	...	...	...	+	...	...	...	Putative histone H2A
At4g07960	0.73	...	...	...	+	...	...	...	Glycosyltransferase family 2
At2g23930	0.72	...	+	...	...	...	...	...	Small nuclear ribonucleo protein E -related
At2g20890	0.72	...	+	...	...	...	...	...	Similar to chloroplast thylakoid formation 1 (<i>A. thaliana</i>) e-163
At4g00430	0.71	+	-	...	...	+	...	...	<i>A. thaliana</i> mRNA for transmembrane protein
At2g21860	0.71	...	+	...	...	...	...	...	Violaxanthin de-epoxidase-related
At2g29440	0.70	...	-	-	...	...	...	...	Putative glutathione transferase
At5g61130	0.70	...	...	...	+	...	...	...	Glycosyl hydrolase family protein 17, similar to β -1,3-glucanase (<i>Camellia sinensis</i>)
At4g24960	0.69	+	...	...	...	...	...	-	Abscisic acid-induced - like protein
At2g42570	0.67	...	...	...	+	+	...	...	Similar to lustrin A-like (<i>Oryza sativa</i> [<i>japonica</i> cultivar-group]) e-116
At2g27970	0.65	...	...	...	+	...	...	...	CDK-subunit 2
At4g38660	0.65	...	...	...	+	...	...	...	Putative thaumatin-like protein
At5g48300	0.65	...	+	...	...	+	...	...	Glucose-1-phosphate adenylyltransferase, small subunit, chloroplast (ADP-glucose pyrophosphorylase) (APS1)
At5g59870	0.65	...	...	...	+	...	...	...	Putative histone H2A
At2g16830	0.64	-	...	...	...	...	...	...	Unknown protein
At2g23120	0.62	+	...	...	...	+	...	...	Unknown protein
At1g22880	0.62	...	...	...	...	...	-	...	Glycosyl hydrolase family 9
At1g13300	0.58	...	...	...	-	...	...	...	Myb family transcription factor
At3g14310	0.57	...	+	+	...	...	...	...	Pectin methylesterase -related
At5g01600	0.57	+	-	-	+	-	-	-	Ferritin 1 precursor
At3g16240	0.57	...	...	...	...	+	...	...	Delta tonoplast integral protein (delta-TIP)
At2g02100	0.50	...	+	...	+	...	...	...	Putative plant defensin protein (PDF2.2)
At2g06850	0.24	...	+	...	+	+	...	...	Xyloglucan endotransglycosylase (ext/EXGT-A1)
Total genes in common	7	55	25	24	22	6	4	...	...
Statistical significance	0.42	0.00	0.0	0.00	0.00	0.25	0.16	...	...

U.S.A.). These cells were maintained in 100 ml of Murashige-Skoog (MS) complete medium (M9500 US Biological) containing naphthalene acetic acid at 4.2 mg/liter and kinetin at 0.02 mg/liter. The cultures were shaken at 140 rpm at 23°C and 10 ml were subcultured every week. Plant cells were inoculated with bacteria 3 days after subculture. An overnight culture of bacteria grown at 29°C was centrifuged and resuspended in MS medium to an optical density at 600 nm = 1.0. Each 100-ml culture of plant cells received acetosyringone (AS) (200 µM) and 1 ml of the bacterial suspension. The mock-inoculated controls received AS and 1 ml of MS medium. Plant cells were collected at 4, 12, 24, and 48 h after inoculation and frozen for later RNA extraction. The *Agrobacterium tumefaciens* strain A348 used is a wild-type oncogenic strain. For GUS expression analysis, plasmid pBISN1 (Narasimhulu et al. 1996) was introduced into strain A348, which then was used to transform *Arabidopsis* cells as described above, in parallel with inoculations done with wild-type A348.

GUS colorimetric (histochemical) analysis and selection of experiments for microarray analysis.

Preliminary experiments indicated that the earliest GUS expression was observed at 2 days. Plant cells inoculated with strain A348(pBISN1) for 2, 3, 4, and 7 days were collected and incubated at 37°C for at least 24 h in GUS-staining solution (X-gluc at 0.5 mg/ml, 10 mM EDTA, 0.5 mM ferricyanide, and 0.5 mM ferrocyanide, in 0.1 M phosphate buffer, pH

Fig. 4. Frankiebox and Telobox motifs in downregulated genes. Sequence logos display the motifs for Frankiebox (top) and Telobox (bottom). Below each motif are corresponding hexameric words whose frequency of occurrence in the downregulated gene set (set, out of 303 up- or downregulated genes) is compared with the genomic set (ref). The *P* of the null hypothesis (no difference) is shown.

Fig. 3. Functional categories of regulated genes according to biological process. “Up” and “down” bars illustrate the percentage of genes displaying the response in the given biological category. The “total” bar provides a comparison with the respective category weight in the genome. Triangles mark the responses that differ significantly from the expectation of randomness.

7.0). Approximately 13% of the transformation experiments resulted in strong GUS expression (assessed visually by staining) at 2 days after inoculation. The majority of experiments resulted in either strong (34%) or intermediate (21%) GUS expression only at 3 days after inoculation. A considerable percentage (21%) of the experiments resulted in very low GUS expression, weakly visible at 4 or more days after inoculation. A small percentage (11%) of experiments did not result in any GUS expression, even at later times after inoculation. Independent inoculations were carried on until four experiments resulting in strong or intermediate GUS expression were obtained. Cells inoculated with strain A348 corresponding to these experiments were selected for RNA extraction and further microarray analysis.

RNA extraction.

Total RNA was extracted from *Arabidopsis* cells using the Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.), according to instructions from the manufacturer for plant material containing high levels of polysaccharides.

Preparation of samples for microarray hybridization.

Labeled cDNA samples were prepared starting with 50 µg of DNaseI-treated *Arabidopsis* total RNA. For reverse transcriptase reactions (30 µl), the RNA was combined with nucleotides (0.5 mM dATP, dCTP, and dGTP; 0.1 mM dTTP; and 0.4 mM amino-allyl dUTP), 5 µg of oligo dT(18), reverse transcriptase (Superscript II; Invitrogen), and other standard reagents following the method recommended by the manufacturer. The cDNA subsequently was coupled to either cyanine-3 (Cy3) or cyanine-5 (Cy5) fluorophores using an established amino-allyl coupling procedure (Fazzio et al. 2001). Cy3- and Cy5-labeled cDNA samples were combined and co-hybridized to the array.

Microarray slide preparation and hybridization conditions.

Microarrays were constructed employing a set of 26,090 70-mer oligo probes commercially available from Qiagen (Alameda, CA, U.S.A.). Each oligo was designed to probe for a unique coding region in the *A. thaliana* genome. Oligo probes were spotted individually onto polylysine-coated microscope slides using an OmniGrid 300 high-precision robotic gridded

(GeneMachines, San Carlo, CA, U.S.A.). Each printed array underwent a series of postprocessing steps prior to use. Briefly, they were humidified and snap dried on a 100°C heat block, UV cross-linked at 450 mJ, and blocked with succinic anhydride and sodium borate in 1-methyl-2-pyrrolidinone. Arrays subsequently were washed twice in deionized water, rinsed with 100% ethanol, and immediately spun dried in a centrifuge at 500 rpm. Arrays were stored under vacuum and screened for imaging artifacts prior to use. Arrays were prehybridized in a 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) solution of 3% powdered milk at 42°C for 1 h, then washed in H₂O and propanol, and centrifuged dry. Arrays were hybridized on a GeneTAC Hyb Station for 16 h at 42°C, followed by washings at 42°C for five cycles in 2× SSC and three cycles in 0.1× SSC, and then centrifuged dry. Arrays were scanned using a GenePix 4000 scanner (Axon Instruments, Union City, CA, U.S.A.). Image analysis was performed using GenePix Pro 3.0.

Microarray data analysis.

Array data were normalized simultaneously for systematic intensity-dependent and spatial variation, as described (Cui et al. 2003). This normalization is an extension of the “lowess” normalization proposed by Yang and associates (2002). Normalization was done in Matlab (The MathWorks, Natick, MA, U.S.A.); all remaining data analysis was done in the “R” statistical computing environment (Yang et al. 2002) using the “siggenes” package in Bioconductor (Yang et al. 2002). Normalized data were analyzed to identify candidate differentially expressed genes by the methodology generally referred to as SAM (Tusher et al. 2001). First, a test statistic was computed for each gene to evaluate the evidence for differential expression. The test statistic is similar to the statistic for the paired *t* test, but with a modified denominator. Second, a reference distribution was estimated by permuting samples among the “mock” and “infected” samples. The number of samples in this experiment permitted complete enumeration of all possible permutations. Finally, the computed test statistic for each gene was compared against the reference distribution. For any chosen critical value for the test statistic, an expected false discovery rate was estimated. Advantages of the SAM approach include protection against false positives through the modified *t*

Table 5. Occurrence of regulatory motifs in the downregulated genes promoters

Genes	Genome	Percent	Exp. (%)	<i>P</i>	Down set	Exp. (%)	<i>P</i>
Total	26,000	...	...	...	188	...	...
With Frankiebox ^a	4,526	17.4	...	...	47	32.7	0.006
With Telobox ^a	5,396	20.7	...	...	32	39.0	0.21
With both	1,163	4.5	3.6	1E-13 ^b	22	8.4 ^c	1.6E-06

^a In proximal upstream 500 bp.

^b *P* of χ^2 distribution for the null hypothesis of no difference between expected (from unrelated sets) and observed.

^c Expected from frequency of co-occurrence in genomic set = 32 × 1,163/5,396.

Table 6. Enrichment of the 500-bp gene-proximal 5' region for the Frankiebox motif

Hexamer	Occurrence of Frankiebox-related hexamer ^a					
	In downregulated gene set			In total <i>Arabidopsis</i> gene set		
	In proximal region of			In proximal region of		
	500 bp	1,000 bp	Enrichment	500 bp	1,000 bp	Enrichment
TGGGCT	104	118	0.88	7,088	11,507	0.62
TGGGCC	132	146	0.90	7,681	10,991	0.70
GGGCTT	86	106	0.81	5,884	9,772	0.60
GGGCCT	67	80	0.84	4,522	6,519	0.69
ATGGGC	112	121	0.93	6,711	10,559	0.64

^a Instances of motif occurrence in the 500- versus 1,000-bp region upstream of the start codon or transcription initiation site (as applicable). Enrichment = number in 500/number in 1,000.

Fig. 5. Candidate regulatory motifs in the promoters of downregulated genes. Top graph: cumulative distribution of Frankiebox (red) and Telobox (yellow) on the 500-bp upstream of the start codon, or transcription start site, when available, of downregulated genes. Bottom maps: the position of each Frankiebox (red) and Telobox (yellow) motif is displayed in the upstream region of each gene.

statistic and lessened reliance on parametric assumptions through permutation testing. The data discussed in this publication have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) and are accessible through GEO Series accession number GSE4116.

Quantitative real-time RT-PCR.

To quantify the amount of specific mRNA transcripts present in various samples, quantitative RT-PCR analysis was performed using the Qiagen (Valencia, CA, U.S.A.) Quantitect Probe RT-PCR kit (cat# 204443), Qiagen custom primers, and Applied Biosystems (Foster City, CA, U.S.A.) Taqman probes designed using Primer Express 1.5. These reagents and kits were used according to instructions from the manufacturer. Briefly, the amplification reaction mixture of 20 μ l contained 50 ng of template RNA, 0.5 μ M each primer pair, 0.2 μ M Taqman probe, and 2 $\times$ Qiagen Quantitect Master Mix. Real-time PCR amplification was performed using a Rotogene PCR machine (Corbett Research, Mortlake, Australia), and PCR products were visualized (by the acquisition of ROX and FAM signals) and analyzed with Rotogene Real-Time Analysis software (Corbett Research). Cycling conditions were as follows: one cycle of 50°C for 30 min and one cycle of 95°C for 15 min, followed by 40 cycles of 94°C for 15 s and 60°C for 60 s.

The following oligonucleotide primers and Taqman probes were employed in these experiments: D16454-5', GCATGTGGTGGGATCAGAAAG; D16454-3', TGAGACGACGCCATTGTTCA; TaqMan probe D16454-6FAM-MGB, TCCGTGACCTTGACG; AF331758-5', TCCCTATTTCTCCCCTGGTTA; AF331758-3', GCTCCACCACCTCCCCTTTCT; TaqMan probe AF331758-6FAM-MGB, AATGGTGACAAATAAG; AL022347-5', GCGGCAGGCTGGTTGA; AL022347-3', TGCCCAATAGTACGCGTCTGT; TaqMan probe AL022347-6FAM-MGB, ACAGGATTGTGTCAACC; AB016875-5', CTGCGTCCCTTGCTCGTT; AB016875-3' TGGCTATGTGTCGTGAACCT; TaqMan probe AB016875-6FAM-MGB, AGCTCAACAGGGCGTT; Y14207-5', TGGCGAGCCCTCCCTAA; Y14207-3', ACCTAAGTCTACAGCTTTTCCACATC; TaqMan probe Y14207-6FAM-MGB, CAAGCTGGTCTTTTGT; AY045776-5', GGAATGTGGGAATGTCCTGATT; AY045776-3', TTCCACGCCGTTAGAACCA; TaqMan probe AY045776-6FAM-MGB, TTTCCCGGTCACGAGGT; U39782-5', TGGTTCCTGGAAAGCGTTTT; U39782-3', TTTTCTCCAAACGC GTGTTG; TaqMan probe U39782-6FAM-MGB, TCGTTACCATGAGCTCG; X99097-5', GCGCAGCTTCAAACACAATG; X99097-3', AGATCCACGCGCACTGAAC; TaqMan probe X99097-6FAM-MGB, CCCCCAAACGGCGAC; AB016877-5', GGTGGCTTGGGAAGCTTGT; AB016877-3', CGTTAAA AACGTTAACCGAGCAA; and TaqMan probe AB016877-6FAM-MGB, AAAGGACCACCCGTCAC.

Analysis of promoter motifs in coregulated gene sets.

The 500- and 1,000-bp region 5' of each gene predicted translational start (TIGR version 5.0, January 2004 release) were obtained from the TAIR database site. The frequency of all possible hexameric motifs in these 5' regions was queried and compared with the frequencies of the same motifs in the total genomic set of 500-bp 5' regions using the motif analysis program of TAIR. Motifs whose analysis yielded a *P* of the null hypothesis (no difference from the total genomic set) smaller than 1×10^{-4} were deemed significant. Significant hexameric motifs then were compared with motifs identified by the MEME and AlignACE programs. Using these two programs, we examined the promoters of the 50 most up- or down-regulated genes and, separately, of the next 50 up- or down-regulated genes. Motifs identified by the alignment carried out by MEME and AlignACE were examined for significance by

scanning the consensus motif sequences with the significant hexamers. Consensus sequences were obtained by submitting aligned sets to the Sequence Logo program. As a parallel approach to establishing significance, the *Arabidopsis* promoter data set was probed for the presence of the Frankiebox and Telobox motifs as defined by the redundant nucleotide code (WWTGGGCTWW and TAGGKTTT, respectively). The identified genes were filtered to eliminate those in which the motifs were further than 500 bp from the start site. The data were handled using Excel (Microsoft Corporation, Redmond, WA, U.S.A.) and charted using JMP (SAS, Cary, NC, U.S.A.).

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LITERATURE CITED

- Alfano, J. R., and Collmer, A. 2004. Type III secretion system effector proteins: Double agents in bacterial disease and plant defense. *Annu. Rev. Phytopathol.* 42:385-414.
- Axelos, M., Bardet, C., Liboz, T., Le Van Thai, A., Curie, C., and Lescure, B. 1989. The gene family encoding the *Arabidopsis thaliana* translation elongation factor EF-1 α : Molecular cloning, characterization and expression. *Mol. Gen. Genet.* 219:106-112.
- Bailey, T. L., and Elkan, C. 1995. The value of prior knowledge in discovering motifs with MEME. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* 3:21-29.
- Bako, L., Umeda, M., Tiburcio, A. F., Schell, J., and Koncz, C. 2003. The VirD2 pilot protein of *Agrobacterium*-transferred DNA interacts with the TATA box-binding protein and a nuclear protein kinase in plants. *Proc. Natl. Acad. Sci. U.S.A.* 100:10108-10113.
- Ballas, N., and Citovsky, V. 1997. Nuclear localization signal binding protein from *Arabidopsis* mediates nuclear import of *Agrobacterium* VirD2 protein. *Proc. Natl. Acad. Sci. U.S.A.* 94:10723-10728.
- Bevort, M., and Leffers, H. 2000. Down regulation of ribosomal protein mRNAs during neuronal differentiation of human NTERA2 cells. *Differentiation* 66:81-92.
- Brencic, A., and Winans, S. C. 2005. Detection of and response to signals involved in host-microbe interactions by plant-associated bacteria. *Microbiol. Mol. Biol. Rev.* 69:155-194.
- Brown, R. L., Kazan, K., McGrath, K. C., Maclean, D. J., and Manners, J. M. 2003. A role for the GCC-box in jasmonate-mediated activation of the *PDF1.2* gene of *Arabidopsis*. *Plant Physiol.* 132:1020-1032.
- Casati, P., and Walbot, V. 2003. Gene expression profiling in response to ultraviolet radiation in maize genotypes with varying flavonoid content. *Plant Physiol.* 132:1739-1754.
- Casey, T. M., Pakay, J. L., Guppy, M., and Arthur, P. G. 2002. Hypoxia causes downregulation of protein and RNA synthesis in noncontracting mammalian cardiomyocytes. *Circ. Res.* 90:777-783.
- Chen, J. Q., Dong, Y., Wang, Y. J., Liu, Q., Zhang, J. S., and Chen, S. Y. 2003. An AP2/EREBP-type transcription-factor gene from rice is cold-inducible and encodes a nuclear-localized protein. *Theor. Appl. Genet.* 107:972-979.
- Christie, P. J., and Vogel, J. P. 2000. Bacterial type IV secretion: Conjugation systems adapted to deliver effector molecules to host cells. *Trends Microbiol.* 8:354-360.
- Cui, X., Kerr, M. K., and Churchill, G. A. 2003. Transformations for cDNA microarray data. *Stat. Appl. Genet. Mol. Biol.* Vol. 2, No. 1, Article 4. Online publication
- Denekamp, M., and Smeekens, S. C. 2003. Integration of wounding and osmotic stress signals determines the expression of the AtMYB102 transcription factor gene. *Plant Physiol.* 132:1415-1423.
- Deng, W., Chen, L., Wood, D. W., Metcalfe, T., Liang, X., Gordon, M. P., Comai, L., and Nester, E. W. 1998. *Agrobacterium* VirD2 protein interacts with plant host cyclophilins. *Proc. Natl. Acad. Sci. U.S.A.* 95:7040-7045.
- Ditt, R. F., Nester, E. W., and Comai, L. 2001. Plant gene expression response to *Agrobacterium tumefaciens*. *Proc. Natl. Acad. Sci. U.S.A.* 98:10954-10959.
- Ditt, R. F., Nester, E., and Comai, L. 2005. The plant cell defense and *Agrobacterium tumefaciens*. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 247:207-213.

- Escobar, M. A., and Dandekar, A. M. 2003. *Agrobacterium tumefaciens* as an agent of disease. *Trends Plant Sci.* 8:380-386.
- Fazzio, T. G., Kooperberg, C., Goldmark, J. P., Neal, C., Basom, R., Delrow, J., and Tsukiyama, T. 2001. Widespread collaboration of Isw2 and Sin3-Rpd3 chromatin remodeling complexes in transcriptional repression. *Mol. Cell Biol.* 21:6450-6460.
- Gelvin, S. B. 2000. *Agrobacterium* and plant genes involved in T-DNA transfer and integration. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51:223-256.
- Gelvin, S. B. 2003. *Agrobacterium*-mediated plant transformation: The biology behind the "gene-jockeying" tool. *Microbiol. Mol. Biol. Rev.* 67:16-37.
- Guan, Y., and Nothnagel, E. A. 2004. Binding of arabinogalactan proteins by Yarrow phenylglycoside triggers wound-like responses in *Arabidopsis* cell cultures. *Plant Physiol.* 135:1346-1366.
- Hammond-Kosack, K. E., and Jones, J. D. 1996. Resistance gene-dependent plant defense responses. *Plant Cell* 8:1773-1791.
- Huang, L., McCluskey, M. P., Ni, H., and LaRossa, R. A. 2002. Global gene expression profiles of the cyanobacterium *Synechocystis* sp. strain PCC 6803 in response to irradiation with UV-B and white light. *J. Bacteriol.* 184:6845-6858.
- Hughes, J. D., Estep, P. W., Tavazoie, S., and Church, G. M. 2000. Computational identification of cis-regulatory elements associated with groups of functionally related genes in *Saccharomyces cerevisiae*. *J. Mol. Biol.* 296:1205-1214.
- Jeffrey, I. W., Bushell, M., Tilleray, V. J., Morley, S., and Clemens, M. J. 2002. Inhibition of protein synthesis in apoptosis: Differential requirements by the tumor necrosis factor alpha family and a DNA-damaging agent for caspases and the double-stranded RNA-dependent protein kinase. *Cancer Res.* 62:2272-2280.
- Kalde, M., Barth, M., Somssich, I. E., and Lippok, B. 2003. Members of the *Arabidopsis* WRKY group III transcription factors are part of different plant defense signaling pathways. *Mol. Plant-Microbe Interact.* 16:295-305.
- Kerr, M. K. 2003. Design considerations for efficient and effective microarray studies. *Biometrics* 59:822-828.
- Kim, H. J., Hyun, Y., Park, J. Y., Park, M. J., Park, M. K., Kim, M. D., Lee, M. H., Moon, J., Lee, I., and Kim, J. 2004. A genetic link between cold responses and flowering time through FVE in *Arabidopsis thaliana*. *Nat. Genet.* 36:167-171.
- Kosugi, S., and Ohashi, Y. 1997. PCF1 and PCF2 specifically bind to cis elements in the rice proliferating cell nuclear antigen gene. *Plant Cell* 9:1607-1619.
- Kosugi, S., Suzuka, I., Ohashi, Y., Murakami, T., and Arai, Y. 1991. Upstream sequences of rice proliferating cell nuclear antigen (PCNA) gene mediate expression of PCNA-GUS chimeric gene in meristems of transgenic tobacco plants. *Nucleic Acids Res.* 19:1571-1576.
- Kosugi, S., Suzuka, I., and Ohashi, Y. 1995. Two of three promoter elements identified in a rice gene for proliferating cell nuclear antigen are essential for meristematic tissue-specific expression. *Plant J.* 7:877-886.
- Lacroix, B., Vaidya, M., Tzfira, T., and Citovsky, V. 2005. The VirE3 protein of *Agrobacterium* mimics a host cell function required for plant genetic transformation. *EMBO (Eur. Mol. Biol. Organ.) J.* 24:428-437.
- Lin, J. F., and Wu, S. H. 2004. Molecular events in senescing *Arabidopsis* leaves. *Plant J.* 39:612-628.
- Ludwig, A., and Tenhaken, R. 2001. Suppression of the ribosomal L2 gene reveals a novel mechanism for stress adaptation in soybean. *Planta* 212:792-798.
- Lui, W. O., Foukakis, T., Liden, J., Thoppe, S. R., Dwight, T., Hoog, A., Zedenius, J., Wallin, G., Reimers, M., and Larsson, C. 2005. Expression profiling reveals a distinct transcription signature in follicular thyroid carcinomas with a PAX8-PPAR(gamma) fusion oncogene. *Oncogene* 24:1467-1476.
- Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K. A., Dangel, J. L., and Dietrich, R. A. 2000. The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. *Nat. Genet.* 26:403-410.
- Manevski, A., Berton, G., Bardet, C., Tremousaygue, D., and Lescure, B. 2000. In synergy with various cis-acting elements, plant interstitial telomere motifs regulate gene expression in *Arabidopsis* root meristems. *FEBS (Fed. Eur. Biol. Soc.) Lett.* 483:43-46.
- Marathe, R., Guan, Z., Anandalakshmi, R., Zhao, H., and Dinesh-Kumar, S. P. 2004. Study of *Arabidopsis thaliana* resistome in response to cucumber mosaic virus infection using whole genome microarray. *Plant Mol. Biol.* 55:501-520.
- May, M. J., and Leaver, C. J. 1993. Oxidative stimulation of glutathione synthesis in *Arabidopsis thaliana* suspension cultures. *Plant Physiol.* 103:621-627.
- Nam, J., Mysore, K. S., Zheng, C., Knue, M. K., Matthysse, A. G., and Gelvin, S. B. 1999. Identification of T-DNA tagged *Arabidopsis* mutants that are resistant to transformation by *Agrobacterium*. *Mol. Gen. Genet.* 261:429-438.
- Narasimulu, S. B., Deng, X. B., Sarria, R., and Gelvin, S. B. 1996. Early transcription of *Agrobacterium* T-DNA genes in tobacco and maize. *Plant Cell* 8:873-886.
- Nester, E. W. 2000. DNA and protein transfer from bacteria to eukaryotes—the *Agrobacterium* story. *Mol. Plant Pathol.* 1:87-90.
- Nimchuk, Z., Eulgem, T., Holt, B. F., 3rd, and Dangl, J. L. 2003. Recognition and response in the plant immune system. *Annu. Rev. Genet.* 37:579-609.
- Pu, X. A., and Goodman, R. N. 1992. Induction of necrogenesis by *Agrobacterium tumefaciens* on grape explants. *Physiol. Mol. Plant Pathol.* 41:241-254.
- Raghavan, C., Ong, E. K., Dalling, M. J., and Stevenson, T. W. 2005. Effect of herbicidal application of 2,4-dichlorophenoxyacetic acid in *Arabidopsis*. *Funct. Integr. Genomics* 5:4-17.
- Redman, J. C., Haas, B. J., Tanimoto, G., and Town, C. D. 2004. Development and evaluation of an *Arabidopsis* whole genome Affymetrix probe array. *Plant J.* 38:545-561.
- Regad, F., Lebas, M., and Lescure, B. 1994. Interstitial telomeric repeats within the *Arabidopsis thaliana* genome. *J. Mol. Biol.* 239:163-169.
- Roberts, R. L., Metz, M., Monks, D. E., Mullaney, M. L., Hall, T., and Nester, E. W. 2003. Purine synthesis and increased *Agrobacterium tumefaciens* transformation of yeast and plants. *Proc. Natl. Acad. Sci. U.S.A.* 100:6634-6639.
- Schenk, P. M., Kazan, K., Wilson, I., Anderson, J. P., Richmond, T., Somerville, S. C., and Manners, J. M. 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proc. Natl. Acad. Sci. U.S.A.* 97:11655-11660.
- Schenk, P. M., Kazan, K., Manners, J. M., Anderson, J. P., Simpson, R. S., Wilson, I. W., Somerville, S. C., and Maclean, D. J. 2003. Systemic gene expression in *Arabidopsis* during an incompatible interaction with *Alternaria brassicicola*. *Plant Physiol.* 132:999-1010.
- Schoof, H., Lenhard, M., Haecker, A., Mayer, K. F., Jurgens, G., and Laux, T. 2000. The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. *Cell* 100:635-644.
- Schrammeijer, B., Risseuw, E., Pansegrau, W., Regensburg-Tuink, T. J., Crosby, W. L., and Hooykaas, P. J. 2001. Interaction of the virulence protein VirF of *Agrobacterium tumefaciens* with plant homologs of the yeast Skp1 protein. *Curr. Biol.* 11:258-262.
- Schrammeijer, B., den Dulk-Ras, A., Vergunst, A. C., Jurado Jacome, E., and Hooykaas, P. J. 2003. Analysis of Vir protein translocation from *Agrobacterium tumefaciens* using *Saccharomyces cerevisiae* as a model: Evidence for transport of a novel effector protein VirE3. *Nucleic Acids Res.* 31:860-868.
- Singh, K. B., Foley, R. C., and Onate-Sanchez, L. 2002. Transcription factors in plant defense and stress responses. *Curr. Opin. Plant Biol.* 5:430-436.
- Smolen, G. A., Pawlowski, L., Wilensky, S. E., and Bender, J. 2002. Dominant alleles of the basic helix-loop-helix transcription factor ATR2 activate stress-responsive genes in *Arabidopsis*. *Genetics* 161:1235-1246.
- Sturm, A., and Chrispeels, M. J. 1990. cDNA cloning of carrot extracellular beta-fructosidase and its expression in response to wounding and bacterial infection. *Plant Cell* 2:1107-1119.
- Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H.-S., Han, B., Zhu, T., Zou, G., and Katagiri, F. 2003. Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *Plant Cell* 15:317-330.
- Tao, Y., Rao, P. K., Bhattacharjee, S., and Gelvin, S. B. 2004. Expression of plant protein phosphatase 2C interferes with nuclear import of the *Agrobacterium* T-complex protein VirD2. *Proc. Natl. Acad. Sci. U.S.A.* 101:5164-5169.
- Tremousaygue, D., Manevski, A., Bardet, C., Lescure, N., and Lescure, B. 1999. Plant interstitial telomere motifs participate in the control of gene expression in root meristems. *Plant J.* 20:5535-61.
- Tremousaygue, D., Garnier, L., Bardet, C., Dabos, P., Herve, C., and Lescure, B. 2003. Internal telomeric repeats and 'TCP domain' protein-binding sites co-operate to regulate gene expression in *Arabidopsis thaliana* cycling cells. *Plant J.* 33:957-966.
- Tusher, V. G., Tibshirani, R., and Chu, G. 2001. Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. U.S.A.* 98:5116-5121.
- Tzfira, T., Vaidya, M., and Citovsky, V. 2001. VIP1, an *Arabidopsis* protein that interacts with *Agrobacterium* VirE2, is involved in VirE2 nuclear import and *Agrobacterium* infectivity. *EMBO (Eur. Mol. Biol. Organ.) J.* 20:3596-3607.
- Tzfira, T., Vaidya, M., and Citovsky, V. 2002. Increasing plant susceptibility to *Agrobacterium* infection by overexpression of the *Arabidopsis* nu-

- clear protein VIP1. Proc. Natl. Acad. Sci. U.S.A. 99:10435-10440.
- Tzfira, T., Li, J., Lacroix, B., and Citovsky, V. 2004. *Agrobacterium* T-DNA integration: Molecules and models. Trends Genet. 20:375-383.
- van Wees, S. C. M., Chang, H.-S., Zhu, T., and Glazebrook, J. 2003. Characterization of the early response of *Arabidopsis* to *Alternaria brassicicola* infection using expression profiling. Plant Physiol. 132:606-617.
- Veena, Jiang, H., Doerge, R. W., and Gelvin, S. B. 2003. Transfer of T-DNA and Vir proteins to plant cells by *Agrobacterium tumefaciens* induces expression of host genes involved in mediating transformation and suppresses host defense gene expression. Plant J. 35:219-236.
- Vergunst, A. C., Schrammeijer, B., den Dulk-Ras, A., de Vlaam, C. M., Regensburg-Tuink, T. J., and Hooykaas, P. J. 2000. VirB/D4-dependent protein translocation from *Agrobacterium* into plant cells. Science 290:979-982.
- Wan, J., Dunning, F. M., and Bent, A. F. 2002. Probing plant-pathogen interactions and downstream defense signaling using DNA microarrays. Funct. Integr. Genomics 2:259-273.
- Wang, Y. J., Zhang, Z. G., He, X. J., Zhou, H. L., Wen, Y. X., Dai, J. X., Zhang, J. S., and Chen, S. Y. 2003. A rice transcription factor OsbHLH1 is involved in cold stress response. Theor. Appl. Genet. 107:1402-1409.
- Wood, D. W., Setubal, J. C., Kaul, R., Monks, D. E., Kitajima, J. P., Okura, V. K., Zhou, Y., Chen, L., Wood, G. E., Almeida, N. F., Jr., Woo, L., Chen, Y., Paulsen, I. T., Eisen, J. A., Karp, P. D., Bovee, D., Sr., Chapman, P., Clendenning, J., Deatherage, G., Gillet, W., Grant, C., Kutayavin, T., Levy, R., Li, M. J., McClelland, E., Palmieri, A., Raymond, C., Rouse, G., Saenphimmachak, C., Wu, Z., Romero, P., Gordon, D., Zhang, S., Yoo, H., Tao, Y., Biddle, P., Jung, M., Krespan, W., Perry, M., Gordon-Kamm, B., Liao, L., Kim, S., Hendrick, C., Zhao, Z. Y., Dolan, M., Chumley, F., Tingey, S. V., Tomb, J. F., Gordon, M. P., Olson, M. V., and Nester, E. W. 2001. The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. Science 294:2317-2323.
- Yang, Y. H., Dudoit, S., Luu, P., Lin, D. M., Peng, V., Ngai, J., and Speed, T. P. 2002. Normalization for cDNA microarray data: A robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res. 30:1362-4962.
- Yokoyama, R., and Nishitani, K. 2001. A comprehensive expression analysis of all members of a gene family encoding cell-wall enzymes allowed us to predict cis-regulatory regions involved in cell-wall construction in specific organs of *Arabidopsis*. Plant Cell Physiol. 42:1025-1033.
- Zhu, J., Oger, P. M., Schrammeijer, B., Hooykaas, P. J., Farrand, S. K., and Winans, S. C. 2000. The bases of crown gall tumorigenesis. J. Bacteriol. 182:3885-3895.
- Zhu, Y., Nam, J., Humara, J. M., Mysore, K. S., Lee, L. Y., Cao, H., Valentine, L., Li, J., Kaiser, A. D., Kopecky, A. L., Hwang, H. H., Bhattacharjee, S., Rao, P. K., Tzfira, T., Rajagopal, J., Yi, H., Veena, Yadav, B. S., Crane, Y. M., Lin, K., Larcher, Y., Gelvin, M. J., Knue, M., Ramos, C., Zhao, X., Davis, S. J., Kim, S. I., Ranjith-Kumar, C. T., Choi, Y. J., Hallan, V. K., Chattopadhyay, S., Sui, X., Ziemienowicz, A., Matthyse, A. G., Citovsky, V., Hohn, B., and Gelvin, S. B. 2003. Identification of *Arabidopsis* rat mutants. Plant Physiol. 132:494-505.

AUTHOR-RECOMMENDED INTERNET RESOURCES

- The Arabidopsis Information Resource (TAIR) database:
www.Arabidopsis.org
- TAIR GO annotation search website:
www.arabidopsis.org/tools/bulk/go/index.jsp
- The National Center for Biotechnology Information Gene Expression Omnibus website: www.ncbi.nlm.nih.gov/geo/