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REVIEW

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# Probing plant-pathogen interactions and downstream defense signaling using DNA microarrays

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**Abstract** The interaction between a plant and a pathogen activates a wide variety of defense responses. The recent development of microarray-based expression profiling methods, together with the availability of genomic and/or EST (expressed sequence tag) sequence data for some plant species, has allowed significant progress in the characterization of plant pathogenesis-related responses. The small number of expression profiling studies completed to date have already identified an amazing number of genes that had not previously been implicated in plant defense. Some of these genes can be associated with defense signal transduction or antimicrobial action, but the functional contribution of many others remains uncertain. Initial expression profiling work has also revealed similarities and distinctions between different defense signaling pathways, and cross-talk (both overlap and interference) between pathogenesis-related responses and plant responses to other stresses. Potential transcriptional *cis*-regulatory elements upstream of co-regulated genes can also be identified. Whole-genome arrays are only now becoming available, and many interactions remain to be studied (e.g. different pathogen species, plant genotypes, mutants, time-points after infection). Expression profiling technologies, in combination with other genomic tools, will have a substantial impact on our understanding of plant-pathogen interactions and defense signaling pathways.

**Keywords** DNA-microarray · Disease-resistance · Expression-profiling · Signal-transduction

## Introduction

Plant-pathogen interactions and downstream signaling are extremely complex and dynamic, and the ongoing interactions between the pathogen and the plant are difficult to monitor with more traditional genetic and biochemical methods. With the advent of the large-scale genomic sequencing and EST (expressed sequence tag) projects, and with the development of DNA microarray technologies, it is now possible to monitor the expression of hundreds or thousands of genes simultaneously. This can be done under different defense-related treatments and over different time points. The technologies open up tremendous opportunities to identify new pathogenesis-related genes, to identify co-regulated genes and the associated regulatory systems, and to reveal interactions between different signaling pathways (Harmer and Kay 2001; Kazan et al. 2001). Cellular or organismal roles can be proposed for gene products with no previously identified function, or added functions can be proposed for previously studied genes. In this review, a brief overview is followed by a first section that discusses how DNA microarrays are being applied to the study plant-pathogen interactions and downstream defense signaling. In the second section we discuss how the information generated from DNA microarrays fits with the current picture of plant defense pathways. We then close by commenting on gaps and possible future directions for research.

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## Overview: plant responses to pathogens

When a plant and a pathogen come into contact, close communications occur between the two organisms (Hammond-Kosack and Jones 2000; Lucas 1998). Pathogen activities focus on colonization of the host and utilization of its resources, while plants are adapted to detect the presence of pathogens and to respond with antimicrobial defenses and other stress responses. Plant and pathogen species are often highly co-evolved, meaning for example that standard plant barriers to microbial infection can be circumvented by particular pathogen species, but also that otherwise successful pathogens can be blocked by the unique adaptive responses of certain plants. As an

infection plays out, the plant's metabolism often represents a shifting mixture of disease resistance responses and disease susceptibility responses.

Interactions between plants and pathogens induce a series of plant defense responses (Hammond-Kosack and Jones 1996). Calcium and other ion fluxes are observed, and protein kinases are activated. Production of signaling compounds such as reactive oxygen intermediates (ROIs), salicylic acid (SA), nitric oxide (NO), ethylene (ETH) and jasmonic acid (JA) causes activation of many downstream responses. Synthesis of antimicrobial peptides, phenolics, flavonoids and other phytoalexin compounds is induced, and cell wall reinforcement responses are activated. A large number of up-to-date reviews are available that provide further information and references on these topics (e.g. Bonas and Lahaye 2002; Dangl and Jones 2001; Dong 2001; Feys and Parker 2000; Fluhr 2001; Glazebrook 2001; Hulbert et al. 2001; Keen et al. 2001; Lam et al. 2001; Martin 1999; Nimchuk et al. 2001; Nümberger and Scheel 2001; Rowland and Jones 2001; Shirasu and Schulze-Lefert 2000; Staskawicz et al. 2001). Not surprisingly, host transcriptional activity is substantially modulated and redirected over the course of such defense responses (Kazan et al. 2001; Maleck et al. 2000; Rushton and Somssich 1998; Scheideler et al. 2002).

An important and heavily studied class of interactions follow the so-called "gene-for-gene" relationship (Flor 1971). When a pathogen-derived avirulence (avr) protein of a virus, bacterium, fungus, nematode or insect is recognized directly or indirectly by the corresponding resistance (R) protein in the plant, the R protein and its associated cellular machinery typically activate defenses that are sufficiently rapid and strong to make the infection unsuccessful (Dangl and Jones 2001; Ellis and Jones 1998). Hence *R* genes form an important "front end" of the plant immune system, and are exploited widely for disease control in crop plants (Crute and Pink 1996; Simmonds and Smartt 1999). To date more than 40 *avr* genes have been identified from plant pathogens, primarily from the bacterial genera *Pseudomonas* and *Xanthomonas* but also from viral and fungal pathogens (Bonas and Lahaye 2002). The few dozen *R* genes that have been cloned to date encode R proteins with conserved structures, and the genomes of *Arabidopsis* and rice (and presumably most other plant species) each carry a few hundred *R* gene homologs (Michelmore 2000). The *R* gene-mediated pathogen surveillance system allows particularly rapid activation of defense responses such as those listed in the previous paragraph. The hypersensitive response (HR), a programmed plant cell death response at the site of pathogen infection, is often associated with gene-for-gene disease resistance (Heath 2000).

A number of the above cellular responses can also be observed during interactions that are not dominated by *R*/*avr*-mediated defense induction. Pathogenic infections that result in notable disease are still constrained by host defense responses. This fact is nicely demonstrated in plants blocked for salicylic acid production: they are much more heavily colonized and damaged than wild-type disease-susceptible controls (Delaney et al. 1994; Gaffney et al. 1993). Infection by a given pathogen can shift both proximal and distal plant tissues to a physiological state of elevated defensiveness against a broad range of pathogen species. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are related but distinct versions of this systemic host response, and they share two components: elevated production of certain antimicrobial compounds, and potentiation of the defense activation machinery so that antimicrobial responses are activated more strongly and rapidly in response to subsequent infections (see the reviews by Dong 2001; Feys and Parker 2000; Glazebrook 2001).

The term "pathogenesis-related protein" (PR protein) was introduced in the 1970s in reference to the proteins that are newly synthesized or present at substantially increased levels after a plant has been infected (Kitajima and Sato 1999; Keen et al. 2001; van Loon and van Kammen 1970). It is a carefully chosen term that reflects a broad scope of possible cellular roles, and its use helps to restrain premature application of the term "defense protein" to a protein that we know only as showing elevated expression during infection. The same issues of premature data interpretation were encountered with the shift to DNA/RNA-based study of "PR genes" more than 20 years ago, and will continue with the genomics-era study of plant responses to infection. A number of the classically defined PR genes do encode proteins such as chitinases, glucanases or defensins that have been shown to carry antimicrobial activity. However, individual PR proteins apparently make only small quantitative contributions to defense, and the contribution will vary depending on the pathogen target. Biotechnology efforts to improve plant disease resistance by enhancing production of particular PR proteins have generally yielded modest results that did not merit commercialization (e.g. Alexander et al. 1993; Broglie et al. 1991). Despite this fact, genes and proteins newly discovered to exhibit pathogenesis-related expression become obvious candidates that must be hypothesized to play a role in defense. It will become apparent below that the pool of candidate PR genes has expanded substantially through use of microarray-based expression profiling, and now includes a wide range of potential signal transduction products such as protein kinases and transcription factors.

## Applications of DNA microarrays to plant-pathogen interactions

### DNA microarrays are an excellent tool for identifying potential defense-related genes

The technology of DNA microarrays is constantly evolving, but widely used methods can be grouped into two basic types: cDNA microarrays and oligonucleotide-based arrays. Detailed reviews of the technology are

**Table 1** A summary of defense-related experiments using DNA microarrays (*SAR* systemic acquired resistance, *SA* salicylic acid, *MeJ* methyl jasmonate, *JA* jasmonic acid)

| Authors and publication year | Array type and organism | Number of genes represented | Major experimental conditions | Number of defense genes identified |
|---|---|---|---|---|
| Baldwin et al. (1999) | Oligonucleotide-based, maize | 1,500 | Fungus *Cochliobolus carbonum* | 117 |
| Nadimpalli et al. (2000) | Oligonucleotide-based, maize | 1,500 | A lesion mimic maize mutant *Les9* | 70 |
| Maleck et al. (2000) | cDNA-based, *Arabidopsis* | ~7,000 | 14 SAR-inducing or -repressing conditions | ~300 |
| Schenk et al. (2000) | cDNA-based, *Arabidopsis* | 2,375 | SA, MeJ, ethylene | 705 |
| Sasaki et al. (2001) | cDNA-based, *Arabidopsis* | 2,880 | MeJ | 41 JA-responsive genes |
| Desikan et al. (2001) | cDNA-based, *Arabidopsis* | ~8,000 | Oxidative stress ($H_2O_2$) | 175 $H_2O_2$-responsive genes |
| Scheideler et al. (2002) | cDNA-based, *Arabidopsis* | 13,000 ESTs | *Pst* DC3000-avrRpt2 | Multiple |
| Chen et al. (2002) | Oligonucleotide-based, *Arabidopsis* | ~8,200 | *Pst* DC3000 +/-avrRpt2, *ES4326* +/-avrRpt2, *nahG*, *pad4–1*, *npr1–1*, *coi1–1*, *ein2–1* | 74 transcription factors and many other genes |
| Cheong et al. (2002) | Oligonucleotide-based, *Arabidopsis* | ~8,200 | Wounding, pathogens, abiotic stresses, plant hormones | Multiple |
| Wan et al. (manuscript in preparation) | Oligonucleotide-based, *Arabidopsis* | ~8,200 | *avrRpt2, avrRpm1, avrPphB, avrRps4*, delivered by *Pst* DC3000 or *Pseudomonas syringae* pv. *glycinea* R4 | ~300 |

available (Aharoni and Vorst 2001; Donson et al. 2002; Duggan et al. 1999; Lipshutz et al. 1999). Further issues regarding DNA microarray design, construction, data analysis and potential applications have been reviewed and will not be discussed here, but potential users are cautioned that the techniques are prone to a number of methodological and interpretive pitfalls (Aharoni and Vorst 2001; Brazma and Vilo 2000; Cheung et al. 1999; Donson et al. 2002; Duggan et al. 1999; Eisen and Brown 1999; Finkelstein et al. 2002; Kazan et al. 2001; Lipshutz et al. 1999; Lockhart and Winzeler 2000; Sherlock 2000; Wisman and Ohlrogge 2000). There are other methods for transcript profiling that may also be used to study plant-pathogen interactions, such as differential display, SAGE (serial analysis of gene expression), and cDNA-AFLP (cDNA amplified fragment length polymorphism; see the reviews by Donson et al. 2002; Glassbrook and Ryals 2001; and the references therein). However, DNA microarray techniques are particularly suitable for monitoring gene expression changes in plants during plant-pathogen interactions, due to their relative simplicity, comprehensive sampling capacity and high throughput (Kazan et al. 2001). The most attractive feature of DNA microarray techniques is that they allow researchers to examine the responses of hundreds or thousands of genes simultaneously during a given treatment. Using these expression profiles, it is possible to identify differentially present mRNA species and to hypothesize potential defense-associated function based on this differential expression.

Recently, a small number of DNA microarray experiments have identified an amazing number of potential defense-related genes (see Table 1 for a brief summary). Although some of these genes have previously been implicated in plant defense responses, most have not. The derived amino-acid sequences of some of these genes have significant similarity to known proteins, but many of the genes encode hypothetical or unknown products.

A few examples are illustrative. Using a maize DNA microarray representing 1,500 maize genes, Baldwin et al. (1999) identified 117 genes that consistently showed altered mRNA expression in maize 6 h after various treatments with the fungal pathogen *Cochliobolus carbonum*. Using a related microarray, Nadimpalli et al. (2000) identified nearly 70 genes having a twofold or more change in mRNA abundance in the lesion mimic maize mutant, *Les9*. *Les9* is characterized by numerous spontaneous chlorotic to necrotic lesions that occur by the 9- to 14-leaf stage, and shows enhanced resistance to *Bipolaris maydis* and elevated expression of defense-related proteins. Many of the differentially expressed genes identified in the *Les9* mutant are defense-related, while the others are unknowns or are not generally understood to be defense-related. One of them, *Zm-hir3*, is implicated in plant cell death through ion channel regulation.

*Arabidopsis* is a particularly well-developed experimental system that has been utilized in a number of studies. In an important early study that examined gene expression changes in *Arabidopsis* under 14 different SAR-inducing or repressing conditions, including a notable focus on plant mutants, Maleck et al. (2000) identified 413 ESTs that appeared to be associated with SAR. This study used a cDNA microarray containing 10,000 ESTs representing approximately 7,000 genes, or 25–30% of all *Arabidopsis* genes. The number of SAR-regulated genes present on their arrays may actually be larger or smaller; unfortunately, no replications of individual treatments were conducted. The researchers applied a threshold of 2.5-fold change in two or more related treatments to identify relevant genes and compen-

sate for this lack of replications. Experimental replication is costly, but is now widely viewed as essential. In particular, "biological replication" is preferred that uses not only separate chip hybridizations but also independent RNA samples obtained from separate plants grown on different dates or in different locations. The related issue of how to calculate the significance threshold (for concluding difference in mRNA level between experimental treatment and control), and at what level of stringency, is common to nearly all microarray studies. There is a clear need to make the primary data for microarray experiments available so that the same data can be analyzed by others using different criteria. Ready availability of raw data will facilitate maximum capture of value from both publicly and privately funded research expenditures.

Schenk et al. (2000), using a targeted microarray containing 2,375 *Arabidopsis* genes, identified 705 genes that were responsive to the fungal pathogen *Alternaria brassicicola* or to the defense-activating signaling molecules SA, methyl jasmonate (MeJ) or ethylene. These 705 genes included 106 genes with no previously described function or homology, along with putative defense-related genes. Sasaki et al. (2001) identified 41 jasmonate-responsive *Arabidopsis* genes of which 5 genes were JA biosynthesis genes, 3 genes were involved in other signaling pathways (ethylene, auxin, and SA), while others had some known defense association, but most were functionally unknown genes. Desikan et al. (2001), using a cDNA microarray representing approximately 30% of the *Arabidopsis* genome, studied regulation of the transcriptome during oxidative stress and identified 175 non-redundant ESTs that are regulated by $H_2O_2$. A substantial proportion of these ESTs have predicted functions in cell rescue and defense processes. Scheideler et al. (2002), using a particularly impressive custom cDNA microarray representing 13,000 randomly chosen ESTs, monitored changes in *Arabidopsis* transcript levels after attempted infection with an avirulent *Pseudomonas syringae* strain at different time points. They found significant changes in the steady state transcript levels of around 680 genes 10 min after inoculation and, by 7 h, a massive shift in the expression pattern of around 2,000 genes representing many cellular processes.

Using the Affymetrix Arabidopsis GeneChip oligonucleotide array representing around 8,200 *Arabidopsis* genes (Zhu and Wang 2000), Chen et al. (2002) monitored mRNA levels of 402 distinct *Arabidopsis* transcription factors under different environmental stresses. They found 74 transcription factor genes whose expression was altered by bacterial pathogen infection and was reduced or abolished in mutants with defects in SA, MeJ, or ethylene signaling. Some of these transcription factors seem very likely to play a role in plant defense signaling pathways. Many other genes responsive to bacteria, fungi, oomycetes or viruses were also identified in this study (Chen et al. 2002). In our own studies using Affymetrix Arabidopsis arrays we have identified approximately 300 genes regulated by one or more *avr* treatment (*avrRpt2*, *avrRpm1*, *avrPphB* and *avrRps4*; Wan et al., manuscript in preparation). Many of these genes encode unknown proteins or are genes with no previously reported defense functions. The differences observed between the four *R/avr* interactions, all arising in the context of the same *Arabidopsis* host genotype and isogenic *P. syringae* pathogen genotypes, emphasize that each *R/avr* combination can elicit a somewhat different host response. The above examples demonstrate that DNA microarrays are a very powerful tool for simultaneously identifying and hypothesizing functions for many genes that may be involved in a complex process, such as plant-defense signaling. From these studies it is apparent that the number of genes involved in pathogenesis-related responses number in the hundreds. One can foresee that many new defense-related genes will be identified in plants as more genomes and ESTs are sequenced, and as DNA microarrays become less expensive and more accessible to researchers.

Other important trends or themes can be discerned from the above studies. Notably, many workers have used custom-made arrays that were the best available technology choice at the time. The quality of the data derived from these custom arrays is likely to be even more variable than it already is with any given standardized technology. For example, on many EST-based arrays 5% or more of the spotted DNAs are mis-identified. In addition, even for arrays that cover the same organism, different and only partially overlapping sets of genes are being analyzed by different research groups when custom arrays are used. This, together with differences in the technology platform and differences in gene annotation, has made comparison of results between studies a substantial challenge that has not yet been adequately addressed.

Decisions regarding array choice are driven not only by data quality but also by the very real issues of equipment availability, gene availability and array/reagent affordability. The relative merit of these factors deserves very careful consideration. In the near future, whole-genome arrays will be available for some plant species. Whole-genome arrays may offer more analytical capacity than researchers desire for some studies, but will reduce the possibility of a misleading bias toward gene sets that are already known. Standardized whole-genome arrays will also enhance the capacity for meta-analyses across different studies. However, cost, gene availability and other issues will continue to force use of custom arrays in many future studies.

Another clear theme brought home upon inspection of the plant-pathogen microarray studies completed to date concerns the wide diversity of conditions that remain to be examined. Within the same plant-pathogen species pairing much can be learned by studying mutants, transgenic strains and other diverse genotypes. Variation of environment, time point examined, and organ or even tissue under study will of course lend additional information. A wide array of plant-pathogen species pairings

merit attention. It is difficult to predict where the transition-point will reside between "enough" and "too much" descriptive microarray work. However, it is important to recognize that, beyond initial description, expression profiling will for many years provide a powerful analytical tool for hypothesis-driven research.

## Data generated from DNA microarray experiments can be used to identify co-regulated genes or "regulons" and *cis*-elements in the promoters of these genes

Data from microarray experiments can be used to identify co-regulated genes or regulons across the data set. Shared expression patterns to some extent imply shared mechanisms for regulation of gene expression, and possible involvement of the genes in a similar function (Eisen et al. 1998; Kazan et al. 2001).

Cluster analyses are in many ways the "heart and soul" of expression profiling. They can allow identification of previously unrecognized genes that are regulated similarly to genes of known significance. They can allow typing of an organism's response into stereotypical classes (example: "this mutant exhibits typical drought stress responses, but an atypical pathogenesis-related response"). They allow workers to observe what is similar and different about the particular responses elicited by different pathogen or plant genotypes, or other varied conditions. There are two basic types of clustering algorithm in common use. The first is the hierarchical "clustergram" that groups genes both by related regulation patterns and by expression amplitudes (Eisen et al. 1998). The other is the "self-organizing map" (SOMs), which generates expression profiles organized by shape (relative mRNA levels in different treatments), essentially independent of amplitude (Tamayo et al. 1999). Both utilize iterative computational methods. Other clustering methods are also available (see the reviews by Altman and Raychaudhuri 2001; Sherlock 2000). Hands-on refinement of computer-generated outputs can improve the utility (intelligibility) of clusters, as can the grouping of quantitative data into a limited number of bins (e.g. "unchanged, up, strongly up, down, strongly down") prior to clustering.

Cluster analysis also offers the tremendous potential to identify specific gene regulatory *cis*-elements and pathways. Once genes have been clustered according to similarity of expression pattern, adjacent DNA sequences (typically the 5′ sequence a few hundred base pairs immediately upstream of the open reading frame) can be investigated for the shared presence of the same known *cis*-elements that bind transcription factors, or can be queried for conserved 5- to 8-bp sequence elements that may represent novel *cis*-regulatory elements. There are many methods available for promoter element analysis, e.g. the web-based MotifSampler (http://www.esat.kuleuven.ac.be/~thijs/Work/MotifSampler.html), PLACE (http://www.dna.affrc.go.jp/htdocs/PLACE/signalup.html), TRANSFAC (http://transfac.gbf.de/TRANSFAC), MEME (http://meme.sdsc.edu/meme/website/meme.html), Align ACE (http://atlas.med.harvard.edu/cgi-bin/alignace.pl), and commercial software such as GeneSpring (Silicon Genetics, Calif.).

Using gene clustering software, Maleck et al. (2000) identified a cluster containing PR-1 (the "PR-1 regulon") that showed similar expression change patterns across different defense-related treatments. The cluster contains 45 ESTs (representing a maximum of 31 different genes), including the genes encoding PR-1, PR-4, GST (glutahione-S-transferase), and PerC (peroxidase C). Because PR-1 gene activation is a reasonably robust molecular marker for SAR, the authors examined upstream DNA sequences of all genes in that cluster. The previously known W-box ("TTGAC") for WRKY transcription factors was statistically over-represented in the PR-1 regulon promoters, with an average of 4.3 copies of this motif per promoter. Furthermore, the W-boxes are often clustered on the promoters of PR-1 regulon genes. The significant overrepresentation of W-box motifs and their clustering on PR-1 regulon gene promoters suggests that WRKY factors are crucial in co-regulation of these genes. WRKY factors constitute a large group of plant-specific transcriptional regulators shown or implicated in pathogen and stress responses (Eulgem et al. 1999, 2000; Yu et al. 2001). There are other regulons identified in the studies by Maleck et al. (2000) with different properties. For example, in one regulon consisting of 55 ESTs, 2 senescence-associated genes, *sen1* and *sen5*, were strongly represented (11 ESTs of *sen1* and 5 ESTs of *sen5*). This result is briefly discussed in the next section.

A cluster consisting of 41 genes that were regulated by bacteria, fungi, oomycetes or viruses was identified by Chen et al. (2002) using the Affymetrix Arabidopsis GeneChip, although lack of replication may be an issue in this study. Further promoter element analysis identified a novel motif (T/C/G)(T/C/G)(A/T)GAC(C/T)T statistically over-represented in this cluster.

Twelve stress-inducible genes were identified as target stress-inducible genes of DREB1A (a transcription factor that controls stress-inducible gene expression) by Seki et al. (2001) in an experiment monitoring the expression pattern of 1,300 *Arabidopsis* genes under drought and cold stresses using a cDNA microarray. Eleven out of 12 DREB1A target genes were found to contain the dehydration-responsive element (DRE) or DRE-related "CCGAC" core motif in their promoter regions.

The above studies demonstrate that gene clustering is a good starting point for identifying co-regulated genes, and for promoter element analysis. This work can foster identification of transcriptional regulators and pathways not only through identification of up-regulated transcription factors, but also through linker-scanning mutagenesis, DNA-binding protein assays, protein-protein interaction studies and other established techniques of transcriptional analysis. However, not every cluster of genes is meaningful, and not every cluster will yield a meaningful promoter motif.

## DNA microarrays can be used to study cross-talk between different pathways

Recently, cross-talk has been documented between different defense response pathways, or between defense responses and other plant responses. Genetic studies not using microarrays have revealed cross-talk between different *R*-gene-mediated pathways (Austin et al. 2002; Azevedo et al. 2002; Feys and Parker 2000; Glazebrook 2001; Nawrath et al. 2002); between defense and senescence (Obregón et al. 2001; Quirino et al. 1999, 2000); between defense and light signal transduction (e.g. Genoud et al. 1998); and between defense and stress signaling (e.g. Cardinale et al. 2002; Logemann and Hahlbrock 2002). Cross-talk between SA and JA signaling pathways has been a particularly prominent theme within the defense signaling field (e.g. Dong 1998; Feys and Parker 2000; Genoud and Métraux 1999; Glazebrook 2001; Reymond and Farmer 1998). The recent use of DNA microarray technologies has now led to a much stronger appreciation of the nature and extent of this cross-talk.

As an example, a surprising level of overlap in the responses activated by different defense-eliciting treatments was revealed in the pioneering work of Schenk et al. (2000). They identified 169 mRNAs that were co-regulated by multiple treatments/defense pathways. The largest set of co-regulated genes (including both up- and down-regulation) was identified in the comparison of SA and JA treatments. Half of the genes induced by ethylene treatment were also induced by JA treatment. These results clearly demonstrated a substantial network of regulatory interactions and co-regulation between different defense signaling pathways, notably between the SA-responsive and JA-responsive pathways, which were previously thought to act primarily in an antagonistic fashion (Schenk et al. 2000).

Maleck et al. (2000) also identified many points of overlap between the responses activated by different defense-eliciting treatments. In our own microarray experiments (Wan et al., manuscript in preparation), we have identified many genes that are co-regulated by two or more different *R*/*avr* interactions. These instances of co-regulation could be thought of as the "uniform aspect" of the plant defense response, and not as "cross-talk". However, the above studies are equally notable for revealing a substantial number of genes that are regulated only by some and not all defense-eliciting treatments. The existence of these gene sets demonstrates the range of possible antagonistic or reinforcing phenomena that arise within the different pathogenesis-related or defense-related responses of plants.

The data from Schenk et al. (2000) also suggested the existence of cross-talk between defense responses and less obviously related pathways. For example, genes encoding chlorophyll A/B-binding proteins (CAB) were up-regulated by the SA treatment, supporting the hypothesis that the SA-mediated signaling pathway exhibits cross-talk with pathways regulated by the phytochrome A/red light response (Genoud and Métraux 1999). Elevated levels of PR gene expression in an *Arabidopsis* phytochrome A and B signaling mutant (*psi2*) also suggest that light signal transduction and PR gene-signaling pathways are connected (Genoud et al. 1998). Stated another way, the findings remind us that what some workers think of as exclusively pathogenesis-related or light-regulated responses in fact can show substantial overlap. In another DNA microarray experiment by Tepperman et al. (2001), stress and defense-related genes were significantly represented in the genes regulated by phytochrome A, supporting cross-talk between stress/defense and light signaling pathways.

The relationship between resistance and senescence has also received increased attention as it was discovered that some genes are activated during both senescence and plant defense (Obregón et al. 2001; Quirino et al. 1999, 2000). Consistent with this, recent microarray data showed that two senescence-associated genes (*sen1* and *sen5*, represented by 11 ESTs) were over-represented in the cluster of genes (consisting of 55 ESTs) induced by certain defense-related treatments (Maleck et al. 2000). Many transcription factors were induced in both senescence and various stresses (including pathogen infection; Chen et al. 2002).

Studies have shown that many genes associated with defense or pathogenesis are also activated in the wounding and other stress signaling pathways, suggesting that there is an extensive cross-talk between the plant defense and wounding/stress pathways (e.g. Bowler and Fluhr 2000; Cardinale et al. 2002; Cheong et al. 2002; Chico et al. 2002; Logemann and Hahlbrock 2002; White 2002). In agreement with this, recent microarray experiments have shown that many genes, including some CDPKs (calcium dependent protein kinases), were regulated by both biotic and abiotic stresses (e.g. Chen et al. 2002; Cheong et al. 2002; Desikan et al. 2001; Kawasaki et al. 2001). Of special interest is that a group of five transcription factors identified by Chen et al. (2002) in a microarray experiment were activated preferentially by both abiotic stress and bacterial infection (Chen et al. 2002). Data from a very recent microarray study by Cheong et al. (2002) further supports the above notion, in which substantial interactions between wounding, pathogen, abiotic stress and hormonal responses have been revealed.

Given the observed overlap in plant transcriptional responses to different stresses, it is particularly difficult to distinguish the true disease resistance responses and separate them from other pathogenesis-induced responses. Note that a gene showing overlapping regulation in response to diverse stresses may still make important contributions to disease resistance. Note also that, while carefully characterized susceptible responses to virulent pathogens might serve as one of the best controls for data evaluation, these tissues are also carrying out resistance responses. Independent tests of gene function (discussed under "Comments and prospects" below) will provide a key route to identification of genes that make

strong contributions to disease resistance. But the observed overlaps in gene regulation remain an informative lesson about cellular signaling and conserved components of plant stress responses.

From the above sections we can see that the small number of plant-pathogen DNA microarray experiments completed to date have already generated substantial information regarding new potential defense genes, *cis*-elements, and cross-talk among different pathways. Scientists now face defense signaling investigations with a broadened and perhaps more humble point of view. Expression profiling information will serve as the starting point for many future studies, some of which are outlined in the final section of this review.

## How does the microarray data fit into the current knowledge of plant defense signaling pathways?

As mentioned in the Introduction, the interactions between a plant and a pathogen often induce a series of defense responses in plants (Hammond-Kosack and Jones 1996). A recent report showed that a dramatic switch from housekeeping to pathogen defense metabolism occurs in *Arabidopsis* after the pathogen infection (Scheideler et al. 2002), and many of the other papers cited in this review also demonstrate substantial reprogramming of gene expression in plants as they deal with pathogen infection. The following section discusses a number of specific areas related to this reprogramming.

### Pathogen gene expression

Close communication occurs when a pathogen comes into contact with a plant, allowing the pathogen to invade the plant and utilize the plant's resources, and allowing the plant to detect the pathogen's presence and activate a series of defense reactions (Keen 2000). The first microarray-based expression profiling studies that monitor how pathogens respond to contact with the plant are only now being published (Okinaka et al. 2002). Similar studies have been carried out for pathogen-animal interactions (e.g., Belcher et al. 2000; Rappuoli 2000). Recently, fungal gene expression during pathogenesis-related development and host plant colonization has been examined using SAGE (Kahmann and Basse 2001; Thomas et al. 2002). Microarray-based studies of pathogen gene expression will form an important component of future work to understand plant-pathogen interactions.

### *R* genes

Although *R* gene products have been shown to play a key role in initiating many plant defense responses, the data from several recent DNA microarray experiments have supported earlier work in not showing dramatic transcriptional regulation of most known *R* genes during plant-pathogen interactions. Exceptions to the above are known. For example, the *R* gene *Xa1* is induced by pathogen infection (Yoshimura et al. 1998), and the *R* gene *N* undergoes relevant alternative splicing over the course of an infection (Dinesh-Kumar and Baker 2000). However, many *R* genes have not shown notable transcriptional regulation. This may be due in part to the fact that many *R* genes exhibit very low transcript levels, and the current DNA microarray techniques are not always sensitive enough to detect low-abundance transcripts and their changes. Another possible reason is that many *R* genes do not need to be regulated, but instead encode the constitutive receptors that regulate the expression of other genes. Alternatively, regulation of *R* gene expression may be more prominent at post-transcriptional levels (e.g. Austin et al. 2002; Azevedo et al. 2002; Nishimura and Somerville 2002). Up-regulation during defense may also be unfavorable because *R* genes might be toxic when expressed at high levels. In spite of the above, the transcripts of some *R* gene analogs (RGA) or partial length RGAs do show significant regulation during defense responses (Meyers et al., submitted for publication). These genes may help to modulate plant defense responses. Additional studies of responses mediated by different *R* genes will be of substantial interest in helping to define the points of overlap and divergence between different *R* gene pathways.

### Defense signal transduction genes

In addition to *R* genes, genetic screening studies have identified a number of key genes, including *EDS1*, *PAD4*, *NDR1*, *PBS1*, *RAR1*, S*GT1*, *EDS5*, and *NPR1*, which are required for the function of some *R* genes or other defense signaling processes (Austin et al. 2002; Azevedo et al. 2002; Feys and Parker 2000; Glazebrook 2001; Nawrath et al. 2002). To date it is not clear that increased transcription of these genes is required for defense responses. The differential requirement of these genes for the function of some but not all *R* genes demonstrates that there is both convergence and divergence of the signaling pathways utilized by *R* genes. Our own microarray data comparing responses mediated by different *R* genes has revealed co-regulated and differentially regulated sets of genes. Study of different *R* gene-mediated responses as they arise in plants mutated for *EDS1, PAD4, NDR1*, etc., will help to define the specific components controlled by these defense signal transduction mediators. However, issues of pleiotropy and redundancy/overlap of function promise to make this a very challenging task.

### Defense response physiology

Defense signaling pathways leading to the activation of antimicrobial responses result from the concerted action

of a number of additional signaling-related molecules, including ions and ion channels, ROIs, NO, SA, JA, ethylene, MAPK/kinases, transcription factors, lipases, proteases, etc. A number of these are considered below.

*Ion fluxes/channels/pumps/$Ca^{2+}$*

Transient changes in the ion permeability of the plasma membrane appear to be a common early event in defense signaling. Upon pathogen recognition, ion channels located in the plasma membrane appear to increase ion fluxes across the membrane and activate downstream defense responses. For example, two $K^+$ channels and elicitor-responsive $Ca^{2+}$ channels in the plasma membrane have been shown to be regulated by defense-related treatments (see the review by Nümberger and Scheel 2001, and the references therein). Recent microarray experiments have identified some ion channel or pump genes that are regulated by defense-related treatments; for example, a plasma membrane $H^+$-ATPase (203B1T7) gene is up-regulated in *cim* (constitutive SAR) mutants and up-regulated in systemic leaves after challenge with *P. syringae* pv. *tomato* DC3000 expressing *avrRpt2* (Maleck et al. 2000; Supplemental Table A, see Electronic Supplementary Material). Among other ions, $Ca^{2+}$ plays an important role in establishing plant defense, probably through $Ca^{2+}$-binding and sensing proteins, such as calmodulin (CaM) and CDPKs (see Bowler and Fluhr 2000; Chico et al. 2002; Lee and Rudd 2002; White 2002). Chelation of $Ca^{2+}$ has been shown to affect ROI and phytoalexin production, mitogen-activated protein kinase (MAPK) activation, and defense gene activation. The genes encoding a CDPK and a CaM-related protein were recently shown to be up-regulated by oxidative stress (Desikan et al. 2001). The results from Yamakawa et al. (2001) indicate that expression levels of individual CaM genes are differentially regulated both transcriptionally and post-transcriptionally in tobacco plants exposed to stresses such as pathogen-induced hypersensitive cell death and wounding.

*Oxidative burst*

Usually, within minutes of contact between a host plant and a pathogen, ROIs such as $O^{2-}$ and $H_2O_2$ are produced in an oxidative burst (Lamb and Dixon 1997). The origin of ROIs has been controversial, but recently a NADPH oxidase has been shown to be responsible for ROI accumulation during some defense responses in *Arabidopsis* (Torres et al. 2002). Because ROIs can cause damage to proteins, lipids and DNA, ROI production and removal must be strictly controlled. Several enzymes, such as superoxide dismutase, catalase, ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase and glutathione reductase, together with ascorbate and glutathione, are responsible for the removal of ROIs (Møller 2001).

Increasing evidence has suggested that the $H_2O_2$ and other ROIs function not only directly as antimicrobial compounds, but also as signaling molecules in plant defense. ROIs are important in activating defense gene expression in adjacent cells and the whole plant, probably in concert with other signaling molecules. For example, $H_2O_2$ has been shown to induce the expression of defense-related genes such as GST, PAL, and MAPKs in plants (Desikan et al. 1998; Grant et al. 2000; Levine et al. 1994). When Desikan et al. (2001) studied *Arabidopsis* gene expression under oxidative stress, they identified 175 non-redundant ESTs that are regulated by $H_2O_2$. Of these, 113 were induced and 62 repressed by $H_2O_2$. A substantial proportion of these ESTs have predicted functions in cell rescue and defense processes; for example, lipoxygenase, peroxidase, GST, various heat-shock proteins, CaM, CDPKs, other kinases, and various transcription factors. The genes encoding catalase 3 (CAT3) and catalase 1 (CAT1) have also been shown to be regulated by defense-related treatments in other DNA microarray experiments (Maleck et al. 2000; Schenk et al. 2000). These studies strongly support the important role of oxidative burst in activating defense genes, and reemphasize the overlap between pathogenesis-related and oxidative stress transcriptional responses.

*Nitric oxide*

As with ROIs, NO apparently plays an important role in the induction of plant defense. Recent studies demonstrate that NO production is increased at the onset of the HR, but this increased production alone is not sufficient to activate the HR. The HR is apparently triggered by the balance of production of NO and ROIs and can be activated through the interaction of NO with $N_2O_2$ generated from $O^{2-}$ by superoxide dismutase (Delledonne et al. 2001; Wendehenne et al. 2001). NO may activate downstream defense signaling by production of cGMP, which probably activates cyclic nucleotide-gated channels leading to $Ca^{2+}$ and $K^+$ influx and downstream gene activation (Wendehenne et al. 2001). So far, no microarray experiments have examined the genes that are regulated by NO.

*SA*

SA has been extensively demonstrated to play a key role in both local resistance and SAR in plants (Alvarez 2000; Ryals et al. 1996). Overproduction of salicylic acid in plants using bacterial transgenes enhances pathogen resistance and defense gene expression (Verberne et al. 2000). Genetic studies have placed SA activity in a feedback loop both downstream and upstream of cell death (Alvarez 2000). The signal transduction pathway downstream of SA leads to the expression of a number of PR genes, such as PR-1 and β-1,3-glucanase (Ryals et al. 1996). Microarray data obtained from the plants treat-

ed with SA or its analog benzothiadiazol (BTH), or carrying SA-elevating mutations, showed the activation of the above-mentioned defense genes and many other genes (Maleck et al. 2000; Schenk et al. 2000). As previously discussed, genetic studies and recent microarray studies have indicated cross-talk between SA-mediated pathways and JA/ethylene pathways, suggesting possible interactions between different plant signaling pathways.

### *JA*

Jasmonates modulate various physiological events such as resistance to pathogens and insects, fruit ripening and senescence. Wounding and MeJ treatment are known to induce the expression of LOX2, AOS, and various other stress-related genes (Creelman and Mullet 1997). The important JA-related microarray work by Schenk et al. (2000) is covered above. Furthermore, data from Sasaki et al. (2001) suggest the existence of a positive feedback regulatory system for JA biosynthesis and also suggest cross-talk between JA signaling and other signaling pathways.

### *Ethylene*

Ethylene is a common stress hormone. Schenk et al. (2000) demonstrated that the genes encoding SAG12 (a senescence-associated cysteine protease), PDF1.1, PDF1.2, chalcone synthase, acc oxidase, lipoxygenase Lox1, and the ethylene receptor ERS2 were up-regulated by ethylene, while other ethylene-associated genes were down-regulated. Ethylene has previously been shown to play roles both in disease resistance and disease susceptibility (Feys and Parker 2000), hence the interplay between defense, senescence and other ethylene-regulated pathways is likely to be particularly relevant (and particularly challenging) to dissect.

### *MAPKs and other kinases*

Recently, MAP kinases and MAPK signaling have been implicated in plant defense and other stress responses (Asai et al. 2002; Bent 2001; Cardinale et al. 2002; Frye et al. 2001; Innes 2001; Petersen et al. 2000; Zhang and Klessig 2001). For example, the gene encoding AtMEK kinase (MAP3Ka; 226N21T7) appeared to be up-regulated in the incompatible interaction caused by *Peronospora parasitica* Noco2 and BTH in *Arabidopsis* Ws-0, but down-regulated by BTH in *Arabidospsis* Col 0 (Maleck et al. 2000; Supplemental Table A, see Electronic Supplementary Material). MAP kinase 3 (ATMPK3) gene was up-regulated by SA, MJ (Schenk et al. 2000), and by oxidative stress (Desikan et al. 2001).

Other genes encoding kinases and phosphatases have been shown to be regulated by defense-related treatments, suggesting probable roles in plant defense. For example, Schenk et al. (2000) showed that receptor protein kinase gene N65832 was up-regulated by SA, and the genes encoding receptor-like protein kinases T44408 and T13648 were down-regulated, respectively, by SA/MJ, and ethylene. Wall-associated kinase 2 (223P15T7) gene appeared to be up-regulated during SAR (Maleck et al. 2000; Supplemental Table A, see Electronic Supplementary Material). The above genes merely represent the "tip of the iceberg." Because protein kinases play key regulatory roles in cell physiology, the kinases that are activated or repressed during plant-pathogen interactions merit careful attention.

### *Transcription factors/DNA-binding proteins*

Transcription factors are also receiving very close attention. The *Arabidopsis* genome encodes more than 1,500 transcription factors (Riechmann et al. 2000) and a number of transcription factor families, such as AP2/EREBP, WRKY, bZIP/HD-ZIP, Myb, and zinc finger proteins, have been implicated in plant stress responses (Shinozaki and Yamaguchi-Shinozaki 2000). Some studies have demonstrated overlap between stress and defense pathways, giving more support to the idea that stress-responsive transcription factors may also play a role in mediating responses to pathogen infection. For example, members of the family of WRKY transcription factors have been implicated in the control of some stress responses (Eulgem et al. 2000). Recent studies show WRKY genes are up-regulated in response to a diverse set of stresses, including infection by pathogens, wounding, and senescence. The induced accumulation of WRKY mRNA is often extremely rapid and appears independent of de novo synthesis of regulatory factors (Eulgem et al. 2000). Recently, the expression of NPR1, which encodes a key regulator of defense responses in *Arabidopsis*, has been shown to be controlled by WRKY factors (Yu et al. 2001).

Schenk et al. (2000) found that the genes encoding a transcription factor-like homeobox-leucine zipper protein, MYB cov1, and AP2 domain-containing protein were up-regulated by various defense-related treatments.

In recent microarray experiments that serve as an excellent model, Chen et al. (2002) specifically studied the regulation of *Arabidopsis* transcription factors under different stresses (including defense-related stresses), and found that the expression of 74 transcription factor genes was responsive to bacterial pathogen infection, and was reduced or abolished in mutants that have defects in SA, JA, or ethylene signaling, suggesting that these transcription factors play an important role in plant defense (Chen et al. 2002).

### *Proteases*

The important role of proteases has perhaps been most strongly demonstrated in the studies of programmed cell

death in animals (Adrain and Martin 2001). Recently, proteolytic enzymes and processes have been implicated or shown to play a regulatory role in plant defense responses and the HR cell (e.g. Krüger et al. 2002; Lam and del Pozo 2000; Nishimura and Somerville 2002). So far the targets for degradation are unknown, but proteolytic enzymes can apparently serve as negative as well as positive regulators of the plant defense response (Austin et al. 2002; Azevedo et al. 2002; Beers et al. 2000; Nishimura and Somerville 2002). Microarray experiments have identified putative cysteine protease genes and other protease genes that are induced by pathogen inoculation, MeJ, ethylene, oxidative stress, and other defense-associated conditions (see studies cited in Table 1).

The above studies have confirmed that plants go through tremendous changes in transcription and/or translation to deal with pathogen infection. The good news is that data from microarray experiments generally correlate well with the findings from other studies, and there are vast amounts of information that can be collected using DNA microarrays. The challenge now is to allocate defined biological functions to individual genes and to achieve an integrated sense of how individual gene products function in pathways and in different types of plant responses.

## Comments and prospects

From the above brief review, we can see that DNA microarrays are a powerful tool in probing plant-pathogen interactions and the downstream signaling pathways. As was stated above, a large amount of information related to defense genes, cross-talk, potential *cis*-elements, etc., has been generated from only a few DNA microarray experiments. However, the following generic points need to be kept in mind when drawing conclusions from any microarray data.

1. DNA microarrays measure the steady-state mRNA abundance and do not measure the rate of transcription. And they certainly do not measure protein abundance, or protein activity levels.
2. The correlation between mRNA and protein levels has appeared in recent studies to be unexpectedly low (Gygi et al. 1999). This low correlation is generally hypothesized to result from post-transcriptional regulation. However, Jansen et al. (2002) postulate the existence of "error pipelines" that overestimate this phenomenon and mask a substantial degree of biologically relevant correlation between transcription and protein levels.
3. Most current DNA microarray technologies do not dependably monitor genes that are expressed transiently, at low levels, or in a small number of cells.
4. cDNA microarrays may not be able to distinguish between gene family members due to high sequence similarities; in this case oligonucleotide-base DNA microarrays may be a better choice.
5. Currently most microarrays only cover part of the corresponding genome.
6. Possible errors exist in sequencing, annotation, sample tracking and handling, array printing, RNA sample preparation and labeling.
7. Replication of experiments is essential, including independent biological replicates.
8. Methods used in data analysis may also affect conclusions; no set standard for data analysis exists and use of different data analysis tools can often lead to different conclusions.

Specific to plant-pathogen interactions, an additional point bears reiteration: As an infection plays out, the plant's metabolism often represents a shifting mixture of disease resistance responses, disease susceptibility responses, and relatively unrelated plant responses that are offshoots of the plant's disease susceptibility and/or defense activation.

Due to the above, caution must be applied when drawing conclusions from microarray data. Both data verification and functional confirmation are usually needed before final conclusions can be drawn. Furthermore, data handling standards need to be established so that results from different microarray experiments can be compared and other researchers can benefit from these data. It would certainly be important and helpful to other researchers if complete sets of raw or minimally processed microarray data were routinely released. Even better is to seek deposition into common databases in standardized formats so that results from different defense-related treatments can be compared. These issues are being addressed (Brazma et al. 2001), but widely accepted solutions have yet to emerge.

For data verification, there are presently two commonly used methods: northern blotting and RT-PCR. Many publications have demonstrated excellent qualitative correlation between northern or RT-PCR work and microarray data (e.g. Kawasaki et al. 2001; Maleck et al. 2000).

### Functional confirmation

To date there is no efficient way to carry out functional confirmation of the identified genes. Identified genes will often have to be verified one by one rather than using high-throughput approaches. For up-to-date information on efforts to streamline this process and generate shared resources, readers are referred to U.S. National Science Foundation documentation (http://www.nsf.gov) concerning Plant Genome projects and the 2010 project (to identify the function of all genes in *Arabidopsis* by the year 2010). Similar efforts are underway in Europe, Japan, China and other countries. The following tools are particularly well suited for "reverse genetic" functional investigation of genes identified in microarray-based studies.

1. Insertional mutagenesis with T-DNA/transposons: for a few plant species such as maize, *Arabidopsis* and

rice, large numbers of knockout lines have been generated using T-DNA or transposon insertional mutagenesis (e.g. Bouché and Bouchez 2001; Kumar and Hirochika 2001; Thorneycroft et al. 2001; Tissier et al. 1999). These are very important resources for future functional analyses of the genes identified through microarray work. For *Arabidopsis*, large sets of sequence-indexed gene knockout lines are being released (see the review by Thorneycroft et al. 2001 and the website http://www.arabidopsis.org/links/insertion.html for relevant resources). One challenge is that many knockouts do not present informative phenotypes that provide a direct clue to gene function (Bouché and Bouchez 2001). However, expression profile information can provide leads that productively focus phenotypic study of gene knockout lines. For example, approximately one-quarter of nearly 2,000 yeast open reading frames disrupted by a transposon exhibit a phenotype when the gene disruption is coupled with expression data and immunolocalization (Ross-Macdonald et al. 1999). Stable gene knockout lines still are not always useful, however, due to the potential for pleiotropic phenotypes, genetic redundancy, physiological compensation, or lethality.

2. Silencing technology: another way to investigate gene function is through gene silencing. This technology will be particularly important for plant species where knockout lines are not available. There are two major methods to silence genes: (1) expression of double-stranded RNA or antisense RNA constructs in transgenic plants (Chuang and Meyerowitz 2000; Smith et al. 2000; Wang and Waterhouse 2001), or (2) expressing a fragment of the target gene using a modified virus vector (virus-induced gene silencing or VIGS; see reviews by Baulcombe 1999; Lindbo et al. 2001; Wang and Waterhouse 2001). The penetrance of gene silencing is variable. Gene silencing efforts typically generate "allelic series" that include many partial loss-of-function plant lines, possibly confusing analysis but also offering an alternative approach when, for example, a total gene knockout is lethal. The generation of stable transgenic silenced plants can be time consuming. For some plant species VIGS may become the more accessible and preferred method (rather than stable transformation with *ds*RNA-generating constructs), but at present VIGS methods have only been established for a few plant species. VIGS is also likely to remain of variable utility given that silencing arises at uneven rates throughout the plant and does not occur in all tissues. However, transient or inducible silencing can offer useful advantages.
3. Transient expression assays: gene overexpression can be as valuable as gene knockout or underexpression in functional studies. Inducible over- or under-expression allows avoidance of many potentially misleading pleiotropic effects that could arise in a plant constitutively and uniformly altered for gene expression. Recently, Kazan et al. (2001) have tested a promising transient expression assay that may be suitable for the high-throughput screening of genes with regulatory functions. In this system, a gene whose function is to be examined is constructed using a constitutive promoter and delivered via microprojectile bombardment into the leaves of "indicator" plants. Indicator plants express a reporter gene under the control of a promoter from a gene (e.g. PR-1 or PDF1.2) which is known to be activated by a specific signaling pathway. Induction of the reported gene expression after bombardment, and not in bombarded controls, suggests the potential involvement of the delivered gene in the relevant signaling pathway. Further approaches are available using chemically inducible gene expression, or gene overexpression via virus vectors (e.g. Aoyama and Chau 1997; Rommens et al. 1995; Scholthof et al. 1993).

Other potentially valuable methods for gene function analysis are less well developed, such as nucleic acid repair using chimeric RNA/DNA oligonucleotides (Oh and May 2001; Rice at al. 2001) and homologous recombination (Puchta 2002). There are of course other powerful "-omics" tools that can be used in studying plant-pathogen interactions, such as proteomics (Kersten et al. 2002) and metabolomics (Fiehn 2002). One can foresee that in the near future DNA microarray technology will be coupled with these tools to compensate for the respective limitations of each method.

DNA microarrays are still very expensive and not accessible to most researchers. Prefabricated arrays are only available for a few plant species, and usually cover only part of the genome. As microarray technology improves and as more ESTs or whole genomes are sequenced, DNA microarrays will become available for more plants and will cover more genes. DNA microarrays will also be available for pathogens to allow gene expression changes to be monitored in both the pathogen and the plant during their interactions. Diagnostic arrays consisting of multiple defense-related genes from one or a few closely related plant species will also be designed so that they can be used at a reasonable cost for diagnostic purposes.

Specific to plant-pathogen interactions and downstream signaling, more microarray studies are likely to be completed that address the following goals:

1. Identify essentially all defense-regulated genes in a plant against a particular pathogen using a whole genome array.
2. Compare spectra of genes affected by different pathogen species, pathovars, isolates, or isogenic strains.
3. Compare spectra of genes affected by the same pathogen in different plant genotypes, including plant mutants that exhibit altered interactions with pathogens.
4. Monitor dynamic gene expression changes over time in a plant from its first contact with a pathogen to the late stages of resistance or disease.
5. Monitor gene expression changes over time in pathogens from their first contact with plants to the estab-

lishment of resistance or disease in the plant, or through the reproductive stages of the pathogen.
6. Compare gene expression at the infection site and in other parts of the plant over time when an infection starts.

To fully exploit the resulting data, this microarray work will generally need to be coupled with the other analytical approaches discussed above.

## Conclusions

Previously, a limited number of resistance genes and downstream defense components had been identified using more traditional molecular genetic and biochemical methods. With these methods it was difficult to examine the coordinated regulation of many genes simultaneously, or the cross-talk between different pathways. DNA microarrays have emerged as a powerful tool that can be used for a genome-wide analysis of plant defense responses after challenge with different pathogens or defense-related treatments. Microarrays are being used to identify potential defense-related genes, to identify potential regulatory *cis*-elements, to characterize and differentiate among the pathways activated during different plant-pathogen pairings, and to examine cross-talk between different pathways or responses. In the future, further microarray studies will be important, as will follow-up studies that functionally examine specific genes or regulatory elements. Much of the information published to date from DNA microarray studies has been consistent with our earlier understanding of host defense, but the substantial and growing body of new expression profile information has generated many new leads and testable hypotheses that will have a strong impact on future research.

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