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Monitoring the Switch from Housekeeping to Pathogen Defense Metabolism in *Arabidopsis thaliana* Using cDNA Arrays*

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Plants respond to pathogen attack by deploying several defense reactions. Some rely on the activation of preformed components, whereas others depend on changes in transcriptional activity. Using cDNA arrays comprising 13,000 unique expressed sequence tags, changes in the transcriptome of *Arabidopsis thaliana* were monitored after attempted infection with the bacterial plant pathogen *Pseudomonas syringae* pv. *tomato* carrying the avirulence gene *avrRpt2*. Sampling at four time points during the first 24 h after infiltration revealed significant changes in the steady state transcript levels of ~650 genes within 10 min and a massive shift in gene expression patterns by 7 h involving ~2,000 genes representing many cellular processes. This shift from housekeeping to defense metabolism results from changes in regulatory and signaling circuits and from an increased demand for energy and biosynthetic capacity in plants fighting off a pathogenic attack. Concentrating our detailed analysis on the genes encoding enzymes in glycolysis, the Krebs cycle, the pentose phosphate pathway, the biosynthesis of aromatic amino acids, phenylpropanoids, and ethylene, we observed interesting differential regulation patterns. Furthermore, our data showed potentially important changes in areas of metabolism, such as the glyoxylate metabolism, hitherto not suspected to be components of plant defense.

As a result of targeted investigations in specific areas of plant biochemistry and physiology, some details of how plants respond to pathogen attack are known (see Refs. 1 and 2). Thus, within minutes of contact between host plant and pathogen, reactive oxygen intermediates are produced in an oxidative burst (3–5). Reactive oxygen intermediates activate defense gene expression in adjacent cells, for example causing transcripts of oxidation stress-protective glutathione *S*-transferases to accumulate, and they can orchestrate the hypersensitive response (HR)[1] (6, 7). The HR is a form of programmed plant cell death occurring in a limited area at the site of infection (8–11). Hypersensitive cell death is complete by 24 h after inoculation with an avirulent pathogen (12). Through the HR, biotrophic pathogens are prevented from establishing a successful nutritional relationship with the host (13). Associated with the HR are a number of other active defense responses, which that are also effective against a wide range of non-biotrophic pathogens. Thus, pathogen spread is hindered by physical strengthening of cell walls through the deposition of polymers such as lignin or callose and oxidative cross-linking of cell wall proteins caused by reactive oxygen intermediates during the HR (4, 14). In addition, phytoalexins, which are newly synthesized secondary metabolites, show direct antimicrobial effects (15). Many highly interconnected signaling networks regulate the initiation of these and other defense reactions. For example, one signaling pathway depends on the phenolic compound salicylic acid, which is essential for the amplification of *R* gene-mediated recognition signals (16). Further signaling components can be plant growth regulators such as ethylene and methyl jasmonate, which have been shown to modulate the defense response in *Arabidopsis* (17–19). These defense reactions are all part of the complex shift in the metabolism of the plant and are necessary for its survival after pathogen attack.

Despite this relatively detailed knowledge in some areas, many aspects of the response of the plant to infection remain uncharacterized. Early attempts to document global changes in, for example, defense-associated gene expression were limited by the difficulty of identifying the significant genes and their products (20). Improvements in technology such as the generation of expressed sequence tag (EST) clones (21) and the complete sequencing of the *Arabidopsis* genome (22–24) offer the potential for a broad understanding of many aspects of plant molecular function. Hybridization of reverse transcripts with arrays of EST clone inserts (25, 26) allow the elucidation of the so-called transcriptome: the whole set of transcripts present at a given time point in a cell, tissue, or organism. For the first time, this technique enables large scale studies on dynamic cellular processes and their regulation and is an important tool in functional genomics (27–29).

In the work reported here, we attempt to relate the changes observed at four time points in the transcriptome of *Arabidopsis* during an HR to the switch from housekeeping to defense metabolism. In the course of this analysis, hundreds of genes representing many cellular processes were identified as differing in their RNA levels. We investigated in detail central metabolic pathways such as glycolysis, the Krebs tricarboxylic acid cycle, the pentose phosphate pathway and other, more specifically defense-related pathways such as the biosynthesis of aromatic amino acids, phenylpropanoids, and ethylene.

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[1] The abbreviations used are: HR, hypersensitive response; hpi, hours post-infection; GST, glutathione *S*-transferase; EST, expressed sequence tag.

EXPERIMENTAL PROCEDURES

Biological Sample Preparation—Arabidopsis thaliana cv. Columbia plants, expressing the resistance gene *RPS2*, were grown in an environmentally controlled growth chamber at constant 22 °C in 8.5 h of daylight. Three of the approximately 12 leaves from 6-week-old plants were infiltrated over approximately two-thirds of their surface area with *Pseudomonas syringae* pv. *tomato* DC3000 carrying the avirulence gene *avrRpt2* on plasmid pABL18 (kind gift from B. Kunkel (30)) resuspended in sterile 10 mM $MgCl_2$ at a concentration of 0.005 A_{600} (corresponding to 5×10^6 colony-forming units ml^{-1}) using a 1-ml syringe without a needle. Control leaves were infiltrated with 10 mM $MgCl_2$ alone. Leaves were harvested within 10 min after infiltration and subsequently 2, 7, and 24 h post-infection (hpi) and then snap-frozen in liquid nitrogen. Leaves were stored at −80 °C until RNA extraction. This regimen was repeated at different dates with at least 15 plants infiltrated for each treatment and time point. To obtain sufficient material for the experiments, samples from replicate experiments were pooled prior to RNA extraction.

EST Clones, PCR Amplification, and cDNA Array Production—A total of 13,800 EST clones were provided by Herman Höfte from Institut National de la Recherche Agronomique (Versailles, France) and the Arabidopsis Biological Resource Center at the Ohio State University (Columbus, OH). A complete list of the clones can be obtained from the authors upon request or can be downloaded from our web page (www.dkfz-heidelberg.de/funct_genome).

To amplify the EST-inserts, PCR reactions were carried out in 384-well microtiter dishes, following the conditions described in detail by Hauser *et al.* (32). Quality of all PCR-products was verified by gel electrophoresis. Clones that could not be amplified with *Taq*-polymerase alone were subjected to a second round of PCR using a long-range extension kit (Roche Diagnostics). In total, 94% (13,002) of the reactions yielded single bands on the gels. Hybridizations of individual fragments to the arrays produced more than one signal. From this, it could be concluded that the clones are not unique but that there is an overall redundancy of 2.5 still contained in the libraries used in this study. All cDNA-clones representing genes that are discussed in this study were confirmed by sequencing.

cDNA arrays were produced by the transfer of PCR products in duplicates to positively charged nylon membranes (Pall Biodyne B, DuPont) using the BioGrid spotting device (BioRobotics, Cambridge, UK) with a 384-pin gadget. To reuse the arrays, the PCR products were fixed onto the membrane by baking and cross-linking.

RNA Extraction and Labeling—Frozen leaf samples were homogenized in the presence of liquid nitrogen. The frozen powder was instantly taken up in RNA-Clean solution (Thermo Hybaid, Ulm, Germany). RNA was extracted following the manufacturer's instructions and purified further by precipitation with 4 M LiCl. First-strand cDNA synthesis and concurrent labeling with ^{33}P-modified nucleotides were performed on total RNA by oligo-dT priming (31, 32). 25–30 μg of total RNA were mixed with 0.5 μg of $(dT)_{15}$ in 11 μl of water. The sample was heated to 70 °C for 10 min and subsequently cooled to 43 °C. Reverse transcription was performed in a total volume of 30 μl using SuperScript II (Invitrogen, Paisley, UK). Incubation was for 1 h at 43 °C in the presence of 0.25 mM each of dATP, dGTP, and dTTP, 1.66 μM dCTP, and 30 μCi of [α-^{33}P]dCTP (3,000 Ci/mmol; Amersham Biosciences, Inc., Buckinghamshire, UK). Subsequently, 1 μl of 1% SDS, 1 μl of 0.5 M EDTA, and 3 μl of 3 M NaOH were added, and the RNA was hydrolyzed for 30 min at 65 °C and 15 min at room temperature. The solution was then neutralized with 10 μl of 1 M Tris-HCl, pH 8, and 3 μl of 2 N HCl. After the addition of 5 μl of 3 M sodium acetate, pH 5.3, 5 μl of tRNA (10 μg/μl), and 60 μl of isopropyl alcohol, the DNA was precipitated at −20 °C for 30 min, pelleted by centrifugation, and taken up in 100 μl of water. Alternatively, the unincorporated nucleotides were removed using QIAquick columns (Qiagen, Hilden, Germany). The incorporation of label was checked by scintillation counting. This procedure was repeated three times, each time using new plant material.

Hybridization—Three independent hybridizations were performed as described in detail earlier (32). The sample was denatured at 100 °C for 5 min or by adding 1/10 volume of 3 M NaOH. Arrays were prehybridized for at least 2 h in 5× SSC, pH 7.5, 5× Denhardt's solution, 0.5% SDS. Hybridization was in the same buffer at 65 °C for 20 h in a volume of 50 μl cm^{-2}, probe concentration being 10–50 Mcpm. Subsequently, filters were rinsed in 2× SSC, 0.1% SDS before being washed for 20 min at 65 °C in the same buffer and again in 0.2× SSC, 0.1% SDS at 65 °C for 1 h. Subsequently, the arrays were exposed to Imaging Plates of a Fuji FLA 3000 (Fuji Film Co., Ltd., Japan) for 48 h.

Data Acquisition and Evaluation—Signal intensities were determined by AIS-Array Vision 4.0 software (Imaging Research, St. Catherines, Ontario, Canada). All 30,720 spots (each cDNA plus appropriate controls being present twice) were identified and quantified after local background subtraction. Raw data quality was evaluated by stringent statistical algorithms described in detail elsewhere (33). Normalization was done by two different methods. In all cases, the overall signal intensity on all spots was used to normalize individual experiments. In addition, spiking probes made from yeast had been added to some samples prior to labeling, permitting an independent cross-check of the results. Routinely, six data points (duplicate spots on each array, three hybridizations per sample) per gene and individual experimental conditions ensured high statistical significance of the results. The median of the data was calculated, and the significance of variations was assessed by two stringency criteria. The highly stringent "min-max separation" is calculated by taking the minimum distance between all data points of the two conditions. The less stringent criterion, called "standard deviation separation," is defined as the difference of the means of either data set diminished by one standard deviation (33). In addition, differential expression was examined if found to be above a ratio threshold of two. Data quality assessment, normalization, and analysis were performed with a software package written in MATLAB (The Mathworks, Natick, MA) (33).

FIG. 1. **Phenotypical response of *Arabidopsis* Col-0 leaves to infiltration.** Leaves depicted in the *upper panel* were infiltrated with 5×10^6 colony-forming units ml^{-1} of *P. syringae* pv. *tomato* + *avrRpt2* and photographed 10 min and 2, 7, and 24 h post-infiltration (from *left* to *right*). The *dark areas* in the leaves to the *left* indicate the infiltrated area. The typical lesions of the HR can be seen in the leaf to the *right* (*arrowheads*). Leaves shown in the *lower panel* were infiltrated with 10 mM $MgCl_2$. Leaves remain *green* and symptom-free.

RESULTS

Infiltration of Avirulent Bacteria Results in Visible Cell Death within 24 h—We infiltrated *P. syringae* pv. *tomato* carrying the avirulence gene *avrRpt2* on a plasmid into leaves of the resistant *Arabidopsis* accession Col-0. Fig. 1 shows the typical changes observed on exemplary leaves within 24 hpi in comparison with mock infiltrated leaves. Formation of necrotic lesions can be microscopically observed ~12 hpi and are clearly visible to the naked eye by 18–24 hpi. (reviewed in Refs. 4, 11, and 34).

Global Changes in Gene Expression—Changes in the transcript levels of *Arabidopsis* after avirulent pathogen attack were catalogued at four time points within the first 24 h after infiltration of the pathogen into leaves. In unrelated experiments, we had encountered differences in transcriptional variation between individual isogenic plants grown under identi-

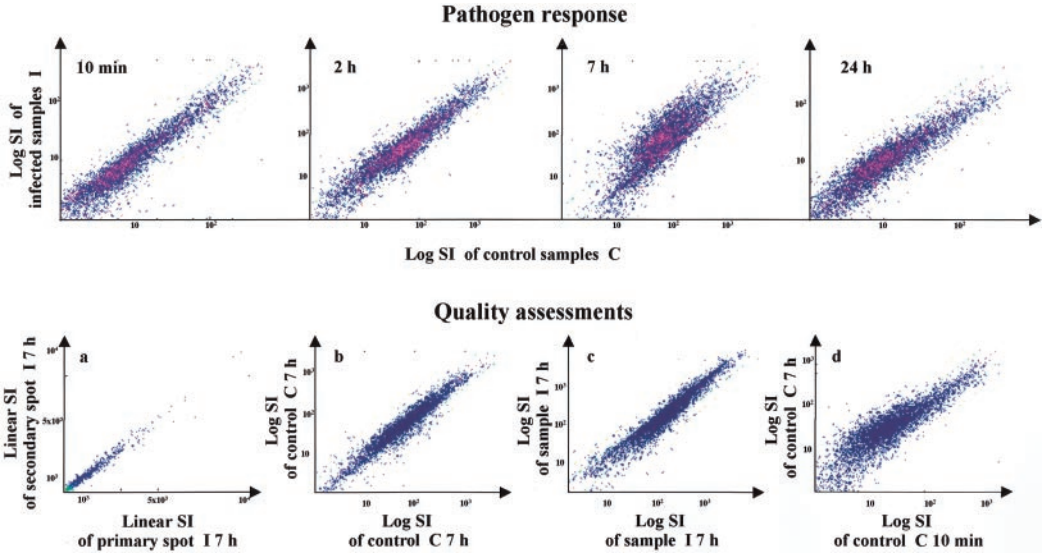

FIG. 2. **Global view of transcript variation upon infection.** *Top row*, comparison of transcript levels of samples from infected plants (*I*) at 10 min and 2, 7, and 24 hpi, respectively, *versus* material isolated from identically treated control plants (*C*) inoculated with $MgCl_2$ only. Each data point represents the mean of signal obtained for an individual gene from the entire set of experiments. The relative *x/y* positions in the scatterplots indicate the respective transcript levels. A location along the diagonal documents a lack of significant variation in transcript levels between infected plant and control. Data points significantly *above* or *below* the diagonal denote up- or down-regulation as compared with the control. Whereas only a relatively small number of changes in transcript levels occurred by 2 hpi, there was a greater response by 7 hpi, which largely had reverted to preinoculation levels by 24 hpi. The results of all 13,000 ESTs for all time points can be downloaded from our web page (www.dkfz-heidelberg.de/funct_genome). *Bottom row*, exemplary control analyses for data obtained from the 7-hpi samples. In *panel a*, the typical signal variation between duplicate spot pairs made from each cDNA is shown. Intentionally, a linear scale was used for this panel, highlighting the fact that the majority of signals are in a lower range of intensity. Because of this, only a statistical analysis permits a comparison of changes across the entire range of intensities. In *panels b* and *c*, signal intensities (*SI*) of individual experiments of the same type (control *versus* control; infected sample *versus* infected sample) are compared; correlation was high at 0.98. In *panel d*, the effects of the 7-h incubation period to the control plants can be seen. The pathogen-specific changes shown above occurred on top of these changes, which reflect circadian and procedural variations. Labeling of axes is in arbitrary units.

cal conditions.[2] Therefore, in the experiments reported here, inoculated leaves from at least 15 plants were sampled in each of the four experiments performed on four different dates, and all similarly treated leaf material was pooled. From this pool of leaves, three separate aliquots of tissue were removed for three separate RNA preparation and labeling reactions. Each labeling reaction product was used in separate array hybridizations. Each array included two duplicate spots of the same EST on the array. Hence for each experimental condition (time point), six data points were generally used in data analysis. The median of the data was calculated, and the significance of variations in RNA levels was assessed by statistical methods (see Ref. 33 and "Experimental Procedures"). The criteria laid down for international standardization and quality control of microarray experiments at the MIAME (minimum information about microarray experiments) conventions (www.mged.org) are achieved or exceeded. Representative sets of results are shown in Fig. 2. Control and pathogen treatments are represented on the *x* and *y* axes, respectively. The tighter the scatter of data points follows the diagonal, the less differential regulation has occurred between treatments. Shifts *above* the diagonal represent up-regulation of a given transcript, whereas shifts *below* the diagonal represent down-regulation. The dramatic shift in steady state mRNA levels is illustrated by the contraction and bunching up of the data points from a relatively tight diagonal at 10 min after infiltration to an irregular ellipse, most pronounced by 7 hpi. By 7 hpi, ~15% of the genes were differentially regulated when compared with the controls. With time, there was an overall increase in transcript levels as documented by the increase in signal intensities. Moreover, initial signal intensities were usually a lot weaker for genes that were down-regulated as compared with signals for up-regulated genes. Thus, relative variation between individual signal measurements for down-regulated genes is higher. Therefore, several such genes might have been "missed" by the analysis procedure because they were evaluated as not showing significant variation according to the stringent quality control criteria applied.

Fig. 3*a* presents the total number of differentially transcribed genes at each time point. Only up- or down-regulation by a factor of 2 or more, which additionally met the significance criteria of at least the standard deviation separation analysis of the signal variations, was scored as significantly differentially regulated. Initially, both plots show a parallel pattern. From the 10-min time point (415 up-regulated and 248 down-regulated transcripts), there was a decline by 2 hpi (299/212) followed by a steep rise at 7 hpi (950/1005). By 24 hpi, the number of up-regulated genes (105) was reduced dramatically, whereas the number of down-regulated genes (927) stayed close to the peak at 7 hpi.

The differentially expressed ESTs shown in Fig. 3*a* were grouped into six functional classes: 1) metabolic enzymes; 2) cellular organization, *e.g.* intracellular transport and cytoskeleton; 3) signal transduction, *e.g.* kinases and phosphatases; 4) control of gene expression, *e.g.* transcription factors, nucleic acid binding proteins, and splicing components; 5) stress responses, *e.g.* those genes with annotations like heat-, salt-, drought-, or cold stress-inducible or *e.g.* peroxidases; and 6) unknown, by far the largest group. When the number of genes in each of the groups (from 1 to 6) are expressed as a percentage of the total number of up- or down-regulated transcripts for a given time point, an interesting kinetic picture emerges (Fig. 3, *b* and *c*). While the proportions of differentially regulated genes in the metabolic enzyme and cellular organization groups show relatively few differences over the 24-h observation period, the number of up-regulated transcripts in the signaling and gene

[2] M. Scheideler and J. D. Hoheisel, unpublished results.

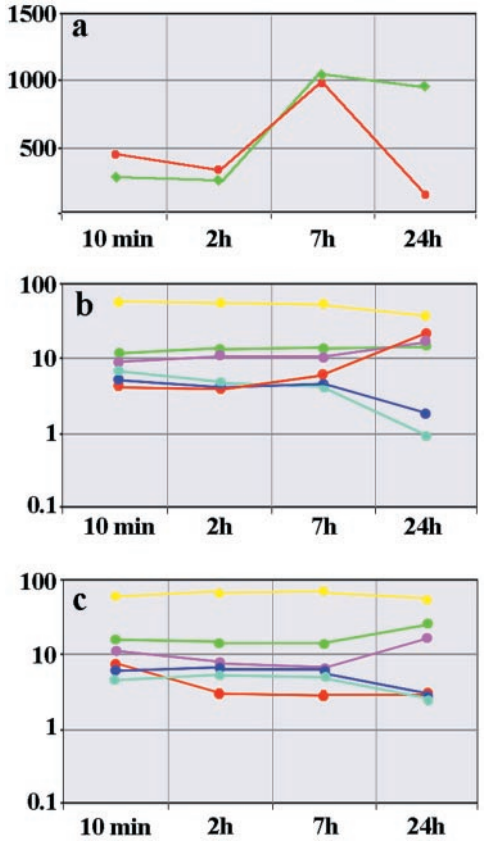

FIG. 3. **Variation in the number of differentially transcribed genes.** In *panel a*, the total number of differentially transcribed genes at the four time points is listed. The number of down-regulated transcripts depicted in *green*; the number of up-regulated genes is depicted in *red*. In *panels b* and *c*, the differentially transcribed genes were grouped into six categories according to function and expressed as the percentage of their numbers relative to the total numbers of up- or down-regulated genes at the respective time point. *Golden*, unknown; *green*, enzymes; *magenta*, cellular organization; *turquoise*, signaling; *purple*, gene expression; *red*, stress response. The list of all the ESTs in the various groups can also be downloaded from our web page (www.dkfz-heidelberg.de/funct_genome).

expression groups decreases markedly from 7 to 24 hpi. Conversely, the number of up-regulated transcripts in the stress response group increases from 6% at 7 hpi to more than 21% by 24 hpi (Fig. 3*b*). Similarly, the down-regulated genes in the metabolic enzyme and cellular organization groups stay at ~10% over the whole 24-h period. The numbers of down-regulated genes in the signaling, gene expression, and stress response categories were initially similar at the 10-min time point at ~7%. This low proportion indicates that very few genes in these groups were immediately down-regulated in response to inoculation. Moreover, this proportion decreases in the stress response group to 3% as early as 2 hpi, whereas the number of ESTs in the other two categories decrease to this level between 7 and 24 hpi (Fig. 3*c*). The list of all the differentially regulated ESTs grouped in the six categories is available from the authors upon request and can be downloaded from our web page.

Dynamics of Metabolism—Physiological reactions known to occur in plants after pathogen attack can be transcriptionally or post-transcriptionally regulated. DNA arrays can only give information about the steady state levels of transcripts at the sampling time; downstream controls are not detected. Thus, it is interesting to follow the 53 genes encoding enzymes of seven metabolic pathways on the array and compare this with what is known from targeted biochemical studies of plants undergoing resistance reactions.

Overall, within the 24-h time period of the investigation, 21 of the 54 selected genes were up-regulated and nine were down-regulated (Fig. 4, Table I). At the first sampling time (10 min after infiltration), only transcripts of the glycolytic enzyme hexokinase (No. 1.1) were up-regulated, whereas glyceraldehyde-3-phosphate dehydrogenase (No. 1.6) was down-regulated, as was coumarate CoA-ligase (No. 5.3) (phenylpropanoid biosynthesis). This differential regulation was transient, and transcript levels of these ESTs had returned to control levels by 2 hpi. Only 1-aminocyclopropane-1-carboxylic acid oxidase (No. 6.3) transcripts from the ethylene biosynthetic pathway were up-regulated at 2 hpi. Thirteen genes were up-regulated by 7 hpi, and transcript levels of all of these had dropped back to control levels by 24 hpi, or in the case for aconitase (No. 2.3), chorismate mutase (No. 4.13), and chalcone synthase (No. 5.4), were down-regulated at this later time point. Only transcripts of glutathione *S*-transferase (GST) were up-regulated throughout the period from 2 to 24 hpi. Interestingly, the transcript levels of phenylalanine ammonia-lyase (No. 5.1) were only marginally up-regulated; the changes however, were not significant under the quality control criteria applied in this investigation. Transcript levels of those genes encoding components of specific defense-related pathways (shikimate, phenylpropanoid, and ethylene biosynthetic pathways and GST) were more likely to show up-regulation (13 out of 27 = 48%) than transcripts representing enzymes on central metabolic pathways (glycolysis, Krebs cycle, pentose phosphate pathway), of which only six out of 25 (or 24%) were up-regulated.

At the 24-h time point, which is after hypersensitive cell collapse has occurred, several of the genes investigated show a down-regulation in the steady state transcript levels, as might be expected. Five were in central metabolism (phosphoglycerate kinase, No. 1.7, aconitase, No. 2.3, glucose-6-phosphate dehydrogenase, No. 3.1, gluconate dehydrogenase, No. 3.3, and transaldolase, No. 3.6) and two (chorismate mutase, No. 4.13 and chalcone synthase, No. 5.4) were in the defense-related pathways. An interesting and to our knowledge novel observation is the up-regulation during a defense response of isocitrate lyase (No. 7.1) and oxalyl-CoA-decarboxylase (No. 7.3), two genes involved in glyoxylate metabolism.

DISCUSSION

Upon recognition of the pathogen by a plant, a series of signaling pathways is activated, leading to *de novo* gene expression that accompanies the activation of the HR cell death program (Fig. 1) and the initiation of several recognized defense responses (35–37). Plant resources become diverted to support this response, which is essential for the evolutionary survival of the plant. Plants do not remain in a perpetual state of defense metabolism, because this would presumably have a too high yield penalty in the long term and might also allow more easy pathogen adaptation. Thus, after execution of a rapid reaction program, the metabolism of the plant returns to normal. However, a state of primed awareness known as systemic acquired resistance remains for some time and enables the plant to respond very rapidly to subsequent pathogen exposure. This envisaged picture of a precipitous shift from housekeeping metabolism to defense metabolism and subsequent return essentially to normal is remarkably well reflected by our DNA array data, particularly considering that we analyzed a complex mixture of infected and responding and adjacent non-infected, non-responding cells. As illustrated in Fig. 2, the initial situation immediately after inoculation and still before delivery of the avirulence factors through the type III secretion system shows few data points not tightly aligned along the diagonal. By 7 hpi, this distribution has changed to show a ballooning out of a large number of the data points from

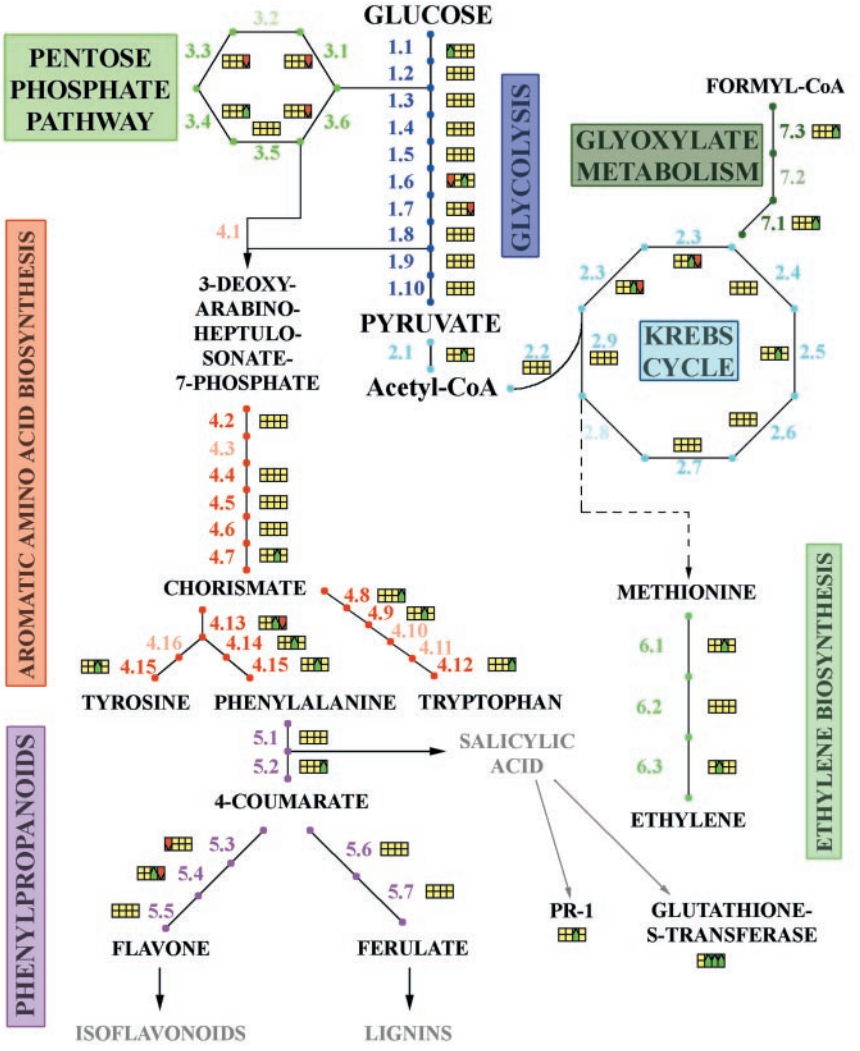

FIG. 4. **Overview of the seven metabolic pathways discussed in the text in detail.** Enzymes whose cDNAs were missing on the array are shown in *light-colored type*. All enzymes are named in Table I. For orientation, selected metabolites are given. Relative changes in steady state transcript levels at each of the sampling times (10 min and 2, 7, and 24 hpi) are indicated in the *boxes*, *left* to *right*: a *yellow* background with a *horizontal line* indicates no significant change, a *green* background and a peak *above* the horizontal shows up-regulation, and a *red* background and a peak *below* the horizontal stands for down-regulation.

the diagonal. By 24 hpi, when the HR cell collapse itself is over, the data point distribution is again nearer to the diagonal.

This pattern is also reflected in the numbers of up- and down-regulated genes at the various sampling times as shown in Fig. 3. In the first 2 h after inoculation, less than 5% of the transcripts showed differential regulation, whereas between 2 and 7 hpi, the proportion increased to ~16% of the ESTs on the array. From 7 to 24 hpi, the proportion of up-regulated genes dropped back to less than 1% of the total, whereas the proportion of down-regulated genes remained quite high at ~7% of the total. This might reflect lower levels of mRNA in general at this time point as compared with the control because the dead HR cells no longer contribute. It might also illustrate that the housekeeping state has not fully returned by 24 hpi after inoculation or that the establishment of the systemic acquired resistance state might require the down-regulation of several genes. Further data from later time points up to and beyond the end of the minimum period necessary for systemic acquired resistance establishment (more than 24 hpi, (12)) could give more credence to this speculation.

When the differentially regulated genes shown in Fig. 3*a* are grouped into classes according to function (see "Results"), specific patterns within the groups become apparent. Thus, while only a low proportion (5%) of the stress response genes (group 5) are up-regulated at the 10-min sampling time, this proportion increases continually over the 24-h period until almost every fifth gene that is up-regulated comes from this group (Fig. 3*b*). Also, *PR-1*, a well known marker gene for pathogenesis-related defense reactions, complies with this scheme, being up-regulated at $t = 7$ hpi. Interestingly, this is rather early because this gene is usually found to be induced by 24 hpi. Other defense-related genes, such as *PR-2*, *PR-5*, or *PDF1.2*, are unfortunately not represented on the array. Between the down-regulated genes (Fig. 3*c*), the proportion of stress response ESTs decreases rapidly from 7% at the 10 min sampling time to 3% by 2 hpi, where it remains throughout. This is compatible with the interpretation that pathogen attack is a stress situation and that the plant up-regulates stress response genes rather than switching them off.

Genes in groups 3 and 4, signaling and control of gene expression, respectively, also show a predictable pattern: the genes in these groups are needed immediately after recognition of the pathogen and for the duration of the defense reaction execution phase. Consequently, the proportion of up-regulated genes stays relatively constant at around 7% for the first 7 hpi but drops to ~1–2% by 24 hpi after HR cell collapse has occurred, and the plant begins to return to a more steady state during which these genes are not so much needed anymore. The number of down-regulated transcripts in classes 3 and 4 remains at around 6% up to 7 hpi and went down to 2% by 24 hpi. (Fig. 3*c*). Obviously, up to 7 hpi, the restructuring of gene expression patterns demands the largest proportional changes in genes in these signaling and control circuits.

Over the 24-h sampling period, the proportion of up- or down-regulated genes in groups 1 and 2 (metabolic enzyme and cellular organization, respectively) remained in the 10–20%

TABLE I

List of names of all enzymes shown in Fig. 3

The fold induction/-repression relative to the control of transcript level is given for each time point. Empty boxes indicate the lack of results due to absence on the arrays of the relevant gene. A comprehensive list of the median of normalized signal intensities of all 13,000 ESTs for all time points can be downloaded from www.dkfz-heidelberg.de/funct_genome.

Pathway	No.	Enzyme Name (Clone ID)	10 min	2h	7h	24h
GLYCOLYSIS	1.1	Hexokinase (201B5T7)	2.0	1.1	1.3	1.4
	1.2	Phosphoglucose isomerase (198H11T7)	1.5	1.3	0.9	1.7
	1.3	Phosphofructokinase (231M11T7)	1.4	1.0	0.9	1.8
	1.4	Aldolase (221P5T7)	1.6	1.2	1.2	0.9
	1.5	Triose phosphate isomerase (179B4T7)	1.1	1.1	1.1	0.7
	1.6	Glyceraldehyde-3-phosphate dehydrogenase (185B10T7)	0.5	0.6	2.3	0.9
	1.7	Phosphoglycerate kinase (108M8T7)	1.2	1.4	1.1	0.5
	1.8	Phosphoglycerate mutase (146O11T7)	0.6	1.0	1.6	1.5
	1.9	Enolase (241P8T7)	0.7	0.9	0.9	1.3
	1.10	Pyruvate kinase (204D3T7)	1.6	1.3	1.2	0.8
KREBS CYCLE	2.1	Pyruvate dehydrogenase (F10F12T7)	1.7	1.0	2.1	1.4
	2.2	Citrate synthase (185J6T7)	1.0	1.0	1.6	0.6
	2.3	Aconitase (177L13T7)	0.9	0.9	3.2	0.5
	2.4	Isocitrate dehydrogenase (165G18T7)	1.2	1.0	1.3	0.6
	2.5	α-Ketoglutarate dehydrogenase (184I14T7)	0.7	0.7	2.4	0.6
	2.6	Succinyl-CoA synthetase (178B19T7)	1.1	1.3	1.8	1.5
	2.7	Succinate dehydrogenase (105N23T7)	1.6	0.8	0.7	1.5
	2.8	Fumarase				
	2.9	Malate dehydrogenase (226H24T7)	0.9	0.8	1.3	0.7
PENTOSE PHOSPHATE PATHWAY	3.1	Glucose-6-phosphate dehydrogenase (70E9T7)	1.4	1.4	1.6	0.5
	3.2	Phosphoglucono lactonase				
	3.3	Gluconate dehydrogenase (VBVBG01)	0.7	1.1	0.8	0.5
	3.4	Ribose-5-phosphate isomerase (171F23T7)	1.2	1.2	1.0	2.0
	3.5	Transketolase (121C14T7)	0.6	0.8	0.8	0.8
	3.6	Transaldolase (221M23T7)	1.6	1.2	0.9	0.5
SHIKIMATE-AROMATIC AMINO ACID BIOSYNTHESIS	4.1	2-Dehydro-3-deoxyphosphoheptanoate aldolase				
	4.2	Dehydroquinate synthase (64B5T7)	1.4	1.3	1.1	1.1
	4.3	Dehydroquinate dehydratase				
	4.4	Shikimate dehydrogenase (98G7T7)	1.7	1.2	0.9	1.2
	4.5	Shikimate kinase (126G8T7)	0.7	0.9	0.9	1.0
	4.6	Shikimate carboxyvinyltransferase (G2C8T7)	1.2	1.1	1.3	1.6
	4.7	Chorismate synthase (143K6T7)	1.0	1.2	2.3	1.6
	4.8	Anthranilate synthase (240I16T7)	1.4	0.8	1.6	2.3
	4.9	Anthranilatephosphoribosyl transferase (172G16)	0.8	0.8	2.0	0.6
	4.10	Phosphoribosylanthranilate isomerase				
	4.11	Indole 3-glycerolphosphate synthase				
	4.12	Tryptophan synthase (F4E1T7)	1.5	1.0	1.8	2.1
	4.13	Chorismate mutase (145F17T7)	0.9	0.8	2.3	0.4
	4.14	Prephenate dehydratase (113G9T7)	1.6	1.2	2.1	1.3
	4.15	Tyrosine transaminase (158C18T7)	0.8	0.8	2.1	0.6
PHENYL-PROPANOIDS	5.1	Phenylalanine ammonium lyase (128N22T7)	1.2	1.2	1.3	0.9
	5.2	Cinnamate monooxygenase (172O5T7)	1.0	1.0	1.3	2.1
	5.3	Coumarate CoA-ligase (G9F10T7)	0.5	0.7	0.7	0.8
	5.4	Chalcone synthase (177N23T7)	1.2	1.1	2.6	0.5
	5.5	Chalcone isomerase (177A20T7)	0.8	1.0	1.2	1.2
	5.6	Hydroxylase (240A11T7)	1.4	0.9	1.5	1.1
	5.7	Methyltransferase (154J19T7)	1.6	1.1	1.5	1.2
ETHYLENE SYNTHESIS	6.1	S-adenosylmethionine synthetase (177J14T7)	1.2	0.8	2.0	1.2
	6.2	ACC synthase (240L12T7)	0.9	1.2	1.3	0.7
	6.3	ACC oxidase (F2A1T7)	1.5	2.0	1.8	0.9
GLYOXYLATE METABOLISM	7.1	Isocitrate lyase (G11H12T7)	0.6	0.8	0.8	2.0
	7.2	Glyoxylate dehydrogenase				
	7.3	Oxalyl-CoA decarboxylase (162G21T7)	0.7	0.6	1.1	2.2

range. This is relatively high as compared with ESTs in groups 3, 4, and 5 and presumably reflects the high metabolic activity and cellular restructuring that accompanies the HR.

In our detailed analysis of seven selected metabolic pathways (Fig. 4, Table I), an interesting picture emerges that is in part compatible and in part at variance with the picture we have of defense metabolism from physiological and biochemical studies. Thus, there is an apparent preponderance for up-regulation of the steady state levels of genes coding for proteins of the stress response pathways with almost 50% of all the enzymes chosen for scrutiny affected, as compared with central metabolic pathways where not even 25% were up-regulated. In this context, it is important to remember that DNA array data only yield information on the steady state levels of particular transcripts, whereas regulation within the cell occurs by several means. Thus, cellular responses are not always manifested through changes in transcriptional activity. For example, it is well known that changes in transcript levels do not necessarily reflect changes in protein concentration or activity within the cell, as with the defense-related transcription factor *NPR1* (38).

With appropriate caution, however, it is still useful to try to interpret our DNA array data in terms of what might be happening in the so-called "metabolome" of the cell after pathogen exposure. Many of the enzymes whose transcript levels were regulated are sequential in a given pathway. Thus, chorismate synthase (No. 4.7), chorismate mutase (No. 4.13), and cinnamate mono-oxygenase (No. 5.2) are located at a branch point of a pathway delivering intermediates feeding into the biosynthesis of tryptophan, tyrosine, or phenylalanine and the subsequent branches of phenolic metabolism. Because of their key positions, it may be that increased transcript levels are important in the regulation of such enzymes to deliver more enzymic protein needed to maintain a high metabolic flux through multiple defense pathways.

One of the advantages of DNA array technology is the potential to screen for new target genes, whose regulation suggests involvement in the process under investigation, in our case plant defense (28, 29). Thus, it was intriguing to note the up-regulation of isocitrate lyase (No. 7.1) and oxalyl-CoA decarboxylase (No. 7.3) transcripts, which might indicate a hitherto unsuspected increase in glyoxylate metabolism associated with plant defense. This possibility is particularly interesting in the light of the recent discovery that *Mycobacterium tuberculosis* is only able to persist in latent infections in macrophages by using isocitrate lyase to enable it to tap fatty acids as carbon and energy sources (40). In an analogous way, the plant may use this pathway to generate energy, allowing carbohydrate intermediates to be diverted for biosyntheses. Mobilization of fatty acids from membrane lipids during the defense reactions of the plant has been observed (39, 40). These fatty acids might be used to generate energy through β-oxidation. Thus, ESTs for enzymes in reactions of the β-oxidation of fatty acids were also investigated. However, no differential regulation of transcript levels of these enzymes was apparent. Nevertheless, our data are indicative of a switch to glyoxylate metabolism, an aspect of defense metabolism that has not previously been identified and that merits further investigation.

Although the application of the powerful new DNA array methodology has allowed us to follow and to some extent interpret the global changes in the transcriptome of *Arabidopsis*

during defense, a both sobering and exciting observation is that 40–60% of the differentially transcribed genes at any given sampling time fall into the unknown category. This illustrates the need for rapid identification and functional analysis of the entire gene set of the model plant *Arabidopsis*. All our data are fully available from www.dkfz-heidelberg.de/funct_genome.

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