Binding of Arabinogalactan Proteins by Yariv Phenylglycoside Triggers Wound-Like Responses in Arabidopsis Cell Cultures

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Arabinogalactan-proteins (AGPs) are cell wall proteoglycans and are widely distributed in the plant kingdom. Classical AGPs and some nonclassical AGPs are predicted to have a glycosylphosphatidylinositol lipid anchor and have been suggested to be involved in cell-cell signaling. Yariv phenylglycoside is a synthetic probe that specifically binds to plant AGPs and has been used to study AGP functions. We treated Arabidopsis suspension cell cultures with Yariv phenylglycoside and observed decreased cell viability, increased cell wall apposition and cytoplasmic vesiculation, and induction of callose deposition. The induction of cell wall apposition and callose synthesis led us to hypothesize that Yariv binding of plant surface AGPs triggers wound-like responses. To study the effect of Yariv binding to plant surface AGPs and to further understand AGP functions, an Arabidopsis whole genome array was used to monitor the transcriptional modifications after Yariv treatment. By comparing the genes that are induced by Yariv treatment with genes whose expressions have been previously shown to be induced by Arabidopsis whole genome array was used to monitor the transcriptional modifications after Yariv treatment. By comparing the genes that are induced by Yariv treatment with genes whose expressions have been previously shown to be induced by other conditions, we conclude that the gene expression profile induced by Yariv phenylglycoside treatment is most similar to that of wound induction. It remains uncertain whether the Yariv phenylglycoside cross-linking of cell surface AGPs induces these genes through a specific AGP-based signaling mechanism or through a general mechanical perturbation of the cell surface.

Arabinogalactan-proteins (AGPs) are widely distributed in plant species and are located at the plasma membrane and cell wall and in the media of cell cultures. These proteoglycans are typically composed of at least 90% carbohydrate by weight. The AGP core polypeptide is usually rich in Hyp, Ser, Thr, and Ala. Extended motifs comparable to those of extensins are not generally found in AGPs, although short stretches of Hyp alternating with Ala or Ser occur in many AGPs. The sugar moieties are composed of (1→3)-β-D-galactan backbones and (1→6)-β-D-galactan side chains with terminal sugars of Ara or GlcUA (Nothnagel, 1997). In the classical AGPs, the nascent polypeptide chain is synthesized with a C-terminal hydrophobic sequence that is later replaced with a glycosylphosphatidylinositol lipid anchor in the mature protein (Gaspar et al., 2001). The Arabidopsis genome contains approximately 47 genes encoding AGP core polypeptides (Schultz et al., 2002).

The abundance of AGP genes and the high degree of posttranslational modifications of AGPs suggest a high genome investment in the synthesis of AGPs, which indicates that these macromolecules have conserved and important roles in plants. Although several possible roles of AGPs have been suggested (Majewska-Sawka and Nothnagel, 2000), the detailed biological functions of AGPs currently remain unknown. Many experiments have demonstrated that the expression of AGPs is developmentally regulated in tissue- and organ-specific manners (Majewska-Sawka and Nothnagel, 2000). Other experiments showed that AGPs are involved in somatic embryogenesis of carrot and in tracheary element redifferentiation of zinnia mesophyll cells (Kreuger and van Holst, 1996; Motose et al., 2001). Recent work with Arabidopsis mutants suggests functions of certain AGPs in cell expansion (Shi et al., 2003), seed germination, in vitro root regeneration (Van Hengel and Roberts, 2003), and response to abscisic acid (Johnson et al., 2003; Van Hengel and Roberts, 2003). Based on the rapid turnover rate of AGPs (Takeuchi and Komamine, 1980; Gibbeaut and Carpita, 1991; Darjania et al., 2002), it has been hypothesized that AGPs may function to prevent aggregation of newly synthesized cell wall polymers in the Golgi and keep these polymers soluble inside secretory vesicles on the way to wall deposition (Gibeaut and Carpita, 1991).

Yariv phenylglycosides such as (β-D-Glc)₃ are synthetic probes that bind and aggregate AGPs. The (β-D-Man)₃ Yariv phenylglycoside differs from the (β-D-Glc)₃ Yariv phenylglycoside only by isomerization of the hydroxyl group at carbon atom 2 of the sugar. Although (β-D-Glc)₃ and (β-D-Man)₃ are extremely close structural analogs, (β-D-Glc)₃ binds AGPs but (β-D-Man)₃ does not, making the latter an excellent control (Yariv et al., 1967; Nothnagel, 1997).
Yariv phenylglycosides are useful not only for purifying AGPs by precipitation but also for perturbing and testing the function of cell surface AGPs in live cells. Perturbation of AGPs using \((\beta-D\text{-Glc})_3\) inhibits cell proliferation in cell cultures (Serpe and Nothnagel, 1994), root growth in Arabidopsis (Willats and Knox, 1996; Ding and Zhu, 1997) and tomato seedlings (Lu et al., 2001), and pollen tube growth in lily (Roy et al., 1998). Treatment with \((\beta-D\text{-Glc})_3\) also induces phenotypic variation in *Streptocarpus prolixus* (Rauh and Basile, 2003).

To elucidate the effects triggered by \((\beta-D\text{-Glc})_3\) and to further understand AGP functions, we used Arabidopsis cell cultures treated with \((\beta-D\text{-Glc})_3\). When applied to Arabidopsis seedlings, \((\beta-D\text{-Glc})_3\) cannot enter the stele, and thus the treatment is only effective at the root epidermal cells (Willats and Knox, 1996).

Fine cell cultures used in the current experiment have the advantage that essentially all cells in the sample receive the treatment. We observed morphological modifications including decreased cell viability, increased cytoplasmic vesiculation, and increased deposition of callose and other polymers at the membrane–cell wall interface. The induction of these cell wall ingrowths including callose synthesis resembled the wound plugs induced by mechanical wounding (Aist, 1976), which led us to hypothesize that \((\beta-D\text{-Glc})_3\)-mediated aggregation of plant cell surface AGPs may trigger wound-like responses. To further examine cellular effects in addition to structural changes, the Arabidopsis whole genome array was used to monitor gene expression during \((\beta-D\text{-Glc})_3\) treatment. Genes with altered expression level were classified into functional groups. The overall pattern of gene expression showed the most resemblance to the previously reported transcriptional profile induced by wounding (Cheong et al., 2002).

**RESULTS AND DISCUSSION**

**Morphological Changes of Cells Treated with \((\beta-D\text{-Glc})_3\) Yariv Phenylglycoside**

The viability of Arabidopsis cell cultures decreased to 50% within approximately 36 h after start of exposure to 50 \(\mu M\) \((\beta-D\text{-Glc})_3\) (Fig. 1). Similar exposure to 50 \(\mu M\) \((\beta-D\text{-Man})_3\), a Yariv phenylglycoside that does not bind AGPs, did not affect cell viability (data not shown). Gao and Showalter (1999) have shown that \((\beta-D\text{-Glc})_3\)-induced loss of viability in Arabidopsis cell cultures occurs via programmed cell death. We observed that callose deposition was detectable by Aniline Blue staining within 6 h after the start of \((\beta-D\text{-Glc})_3\) treatment and increased up to at least 36 h (Fig. 2). Treatment with 50 \(\mu M\) \((\beta-D\text{-Man})_3\) did not induce callose deposition (data not shown). Callose, a \((1\rightarrow3)\)-\(\beta-D\text{-glucan}, is not usually present in plant cells except in phloem sieve plates, pollen tubes, cell plates during cytokinesis, and wounded plant tissues (Kauss, 1996).

![Figure 1. Effect of \((\beta-D\text{-Glc})_3\) on viability of Arabidopsis cells, as monitored by fluorescein diacetate staining. At time 0 h, suspension culture cells were transferred to either 50 \(\mu M\) \((\beta-D\text{-Glc})_3\) in fresh B5 medium (●) or fresh B5 medium alone as the control (○). Experiments were repeated at least four times. Bars indicate SD.](Image 334x566 to 526x713)

The \((\beta-D\text{-Glc})_3\)-treated cells also showed ultrastructural changes including increased intracellular vesiculation and cell wall apposition (data not shown). The increased callose synthesis and cell wall apposition resembled wound plugs (Aist, 1976), which led us to hypothesize that \((\beta-D\text{-Glc})_3\) binding of cell surface AGPs triggers wound-like responses.

**Overview of Gene Expression Changes Resulting from \((\beta-D\text{-Glc})_3\) Treatment**

To further test the hypothesis that Yariv treatment triggers wound-like responses, we used the whole Arabidopsis genome microarray to assess changes in mRNA accumulation. We chose two time points after the start of \((\beta-D\text{-Glc})_3\) treatment, the first early at 1 h and the second somewhat later at 10 h. Because of the onset of cell death in the cultures (Fig. 1), we reasoned that mRNA quality and the interpretability of the results would be compromised at later times. We also imposed a threshold of at least a 2-fold change in expression level when screening for genes with induced or repressed expression. By this criterion, 411 genes were induced (Tables I and II; Supplemental Table I, which can be viewed at www.plantphysiol.org) and 63 genes repressed at 1 h (Supplemental Table II) of \((\beta-D\text{-Glc})_3\) treatment, and 305 genes were induced (Table III; Supplemental Table I) and 369 genes were repressed at 10 h (Supplemental Table III) of \((\beta-D\text{-Glc})_3\) treatment. The induction at 1 h seemed transient for the vast majority of genes since only 25 of the 411 genes induced at 1 h were also among the 305 genes induced at 10 h of \((\beta-D\text{-Glc})_3\) treatment (Supplemental Table IV). A similarly limited overlap of early and late inductions was observed by Cheong et al. (2002) in a study of wounding. Housekeeping genes and cell cycle regulation genes, such as tubulin, kinesin, dynein, cyclin, and histone genes, were generally downregulated at 10 h. Numerous genes involved in cell
Wall synthesis and modification were also down-regulated at 10 h (Supplemental Table III). The relative abundance of down-regulated genes and repression of various housekeeping genes at 10 h were reflective of a general down-turn in cellular activities during ongoing cell death.

Judging from the apparent link between ongoing cell death and gene repression, we decided to principally focus this report on up-regulated genes (Tables II and III) since these, rather than down-regulated genes, might give more valuable information about (β-D-Glc)_3-induced responses and AGP functions. We also focused this report on genes annotated with known or putative functions. Unknown genes that were up-regulated at 1 h and 10 h of (β-D-Glc)_3 treatment can be reviewed elsewhere (Supplemental Table I). All of the fold changes appearing in the tables here were derived by comparing expression levels in (β-D-Glc)_3-treated cultures with expression levels in mock-treated cultures in B5 medium. As an additional control, a microarray experiment was performed with a 1-h (β-D-Man)_3 treatment. While 50 μM (β-D-Glc)_3 induced 410 genes within 1 h of treatment, 50 μM (β-D-Man)_3 induced only 44 genes and down-regulated 20 genes, the majority of these changing only slightly more than 2-fold in expression (Supplemental Table V). Of these 64 genes changed in expression by (β-D-Man)_3, 20 were also changed in expression by (β-D-Glc)_3. With the exception of ZAT11 (see section below on transcription factors), these 20 genes with overlapping expression were excluded from all other tables.

**Genes Induced during Senescence Were Up-Regulated by (β-D-Glc)_3 Treatment**

Arabidopsis cell death triggered by (β-D-Glc)_3 treatment has been suggested to be a form of programmed cell death (Gao and Showalter, 1999). Several genes previously reported to be expressed during plant senescence, a form of programmed cell death, were also induced by (β-D-Glc)_3 treatment (Tables II and III). These genes included those with sequence similarities to senescence-associated genes DSA5 (At2g23810,
Table 1. Summary of genes with mRNA accumulation increased or decreased at least 2-fold at 1 h or 10 h of \( \beta\text{-d-Glc}_3 \) treatment compared to B5 medium control

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At3g45600; Panavas et al., 1999) and SAG21 (At4g02380; Weaver et al., 1998), an Fe(II)/ascorbate oxidase (SRG1; At1g17020; Callard et al., 1996), a glyoxalase II (SAG28; At1g53580; Quirino et al., 1999), and a hin1 homolog (YL39; At2g35980; Pontier et al., 1999; Yoshida et al., 2001). Also induced by \( \beta\text{-d-Glc}_3 \) treatment was a cytochrome p450 (CYP76C2; At2g45570) whose expression had been previously shown to be elevated by senescing of leaves, aging of cell cultures, and wounding of Arabidopsis (Godiard et al., 1998; Yoshida et al., 2001).

Cell Wall-Related Genes with Expression Altered by \( \beta\text{-d-Glc}_3 \) Treatment

Treatment with \( \beta\text{-d-Glc}_3 \) induced deposition of matrix material between the plasma membrane and cell wall of Arabidopsis cells (Fig. 2; other data not shown). This cell wall apposition, which increased with time and involved callose (Fig. 2), was morphologically similar to wound plugs induced by mechanical wounding and to papillae induced by fungal infection (Aist, 1976). Other cell wall changes, specifically bulging of root epidermal cells, have been observed in Arabidopsis seedlings treated with \( \beta\text{-d-Glc}_3 \) (Willats and Knox, 1996; Ding and Zhu, 1997). Root cell wall bulging was also recently reported for Arabidopsis with a mutation in an AGI-like gene (Shi et al., 2003). Cell bulging was not apparent in our \( \beta\text{-d-Glc}_3 \)-treated Arabidopsis culture cells (Fig. 2), and we have previously shown that cell volume does not appreciably change in \( \beta\text{-d-Glc}_3 \)-treated rose culture cells (Serpe and Nothnagel, 1994). Due to the variability in the shapes of Arabidopsis cells in culture (Fig. 2), however, a small amount of bulging would have been difficult to detect. Neither the biochemical changes responsible for cell wall bulging nor the identity of other cell wall components in addition to callose in the paramural deposits (Fig. 2) are known. Identification of cell wall-related genes induced by \( \beta\text{-d-Glc}_3 \) may shed light on both of these issues.

The xyloglucan endotransglycosylases/hydrolases (XTH) can cleave xyloglucan molecules, form a polysaccharide-enzyme intermediate, and then transfer the newly cleaved xyloglucan molecule to the nonreducing end of another xyloglucan polymer (Campbell and Braam, 1999; Rose et al., 2002). The XTHs are proposed to function in cell wall biogenesis, cell wall loosening leading to cell expansion (Vissenberg et al., 2000; Kaku et al., 2002), and cell wall degradation (Redgwell and Fry, 1993; Antosiewicz et al., 1997). The XTH genes can also be induced by hormone and environmental stimuli (Rose et al., 2002). Seven XTH genes were up-regulated at 1 h of \( \beta\text{-d-Glc}_3 \) treatment (Table II). No XTH genes were up-regulated at 10 h. Three of the induced XTH genes, \( At\text{-XTH17 (XTR1), At\text{-XTH22 (TCH4), and At\text{-XTH23 (XTR6)}), were previously shown to be induced by wounding (Table IV; Cheong et al., 2002). Expansins form another class of proteins involved in cell wall loosening and cell extension (Cosgrove et al., 2002). Three genes of this class, \( At\text{EXP12, EXP2, and EXP3}, were induced slightly more than 2-fold at 1 h of \( \beta\text{-d-Glc}_3 \) treatment.

Pectin and pectin changes affect cell wall strength, cell wall porosity, cell wall ion-exchange capacity, cell adhesion, and other aspects of plant development and pathogen response (Micheli, 2001; Willats et al., 2001). Pectin is also deposited in wound plugs (Russo and Bushnell, 1989). As synthesized in the Golgi, pectin is highly methyl-esterified. Later, upon delivery to the cell wall, pectin is partially deesterified by pectin methylesterases (PMEs). A direct molecular effect of this deesterification is the exposure of an ionizable carboxyl group on galacturonosyl residues, which enables the pectin to be stiffened by ionic cross-bonding with Ca\(^{2+}\). Downstream effects of PMEs occur in pectin assembly and disassembly (Willats et al., 2001), tissue integrity (Tieman and Handa, 1994), stem elongation (Pilling et al., 2000), cell adhesion, and cell wall metabolism (Wen et al., 1999). Four pectin esterase genes were induced at 1 h of \( \beta\text{-d-Glc}_3 \) treatment (Table II). Taken together, the induction of XTH, expansin, and PME genes implies the possible modifications of cell wall composition and properties in the treated Arabidopsis cell cultures.

Several \( \beta\text{-1,3-glucanase genes were induced at either 1 h or 10 h of \( \beta\text{-d-Glc}_3 \) treatment. The 22-fold increase in expression of 1 \( \beta\text{-1,3-glucanase gene (At3g04010) was the strongest induction observed on the entire microarray at 10 h (Table III). Many \( \beta\text{-1,3-glucanases are involved in plant defense (Keen and Yoshikawa, 1983; Sela-Buurlage et al., 1993) or development (Buccigalia and Smith, 1994; Delp and Palva, 1999; Buchner et al., 2002), and some \( \beta\text{-1,3-glucanases are induced by wounding or hormone.} \)
<table>
<thead>
<tr>
<th>Probe Set No.</th>
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<th>Gene Description</th>
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**Cell Wall**

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treatment (Simmons et al., 1992; Cheong et al., 2002). Callose ($\beta$-1, 3-glucan) accumulation was evident within 6 h of ($\beta$-D-Glc)$_3$ treatment (Fig. 2), and the induction of $\beta$-1,3-glucanase genes at 1 h and especially 10 h might suggest action of these enzymes in turning over the deposited callose.

In view of the substantial accumulation of callose (Fig. 2), it is interesting that none of the 12 identified callose synthase genes were up-regulated more than 2-fold by ($\beta$-D-Glc)$_3$ (Table V). Two laboratories (Jacobs et al., 2003; Nishimura et al., 2003) have recently shown that one callose synthase, CalS12 (also called GSL5; At4g03550), is required for callose deposition in wound plugs and fungal-induced papillae. Although CalS12 was not induced by ($\beta$-D-Glc)$_3$, the signal reporting the expression of this gene was relatively strong in our cell culture system at 1 and 10 h, with and without treatment (Table V). To test if transcription of the CalS12 gene was transiently up-regulated earlier than 1 h, we used real-time PCR to measure transcript levels at 10 min after the start of treatment. Relative to the mock treatment control, the CalS12 transcript levels were 0.8 ± 0.48 (average of 3 trials ± s.d) for the ($\beta$-D-Glc)$_3$ treatment and 1.36 ± 0.69 for the ($\beta$-D-Man)$_3$ treatment, i.e. no significant induction of CalS12 occurred at 10 min. It remains possible that a callose synthase other than CalS12 is involved in ($\beta$-D-Glc)$_3$-induced callose deposition. To resolve this point, it would be interesting to test if ($\beta$-D-Glc)$_3$ induces callose deposition in a CalS12 knockout plant.

Overall, however, the observed general lack of induction of callose synthase genes (Table V) may indicate that callose synthase activity is regulated posttranscriptionally. Activity of callose synthase protein has been suggested to be regulated by G-proteins (Hong et al., 2001) and Ca$^{2+}$ (Sclupmann et al., 1993; Li et al., 1997; Verma and Hong, 2001), so it is possible that the observed ($\beta$-D-Glc)$_3$-induced callose deposition

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

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Table III. Known genes up-regulated at least 2-fold at 10 h of 50 μM (β-D-Glc)₃ treatment (see Table I caption for other details)

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Responses to Binding of Arabinogalactan Proteins

Cell Rescue, Defense, Cell Death, and Aging

Biogenesis of Plasma Membrane

Cell Wall

Cytoskeleton

Plant Development

Table continues on following page.
<table>
<thead>
<tr>
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<th>Fold Increase</th>
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**Cellular Communication/Signal Transduction**

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<td>At3g57040</td>
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**Organization of Chromosome Structure**

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**Ionic Homeostasis**

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<td>Metallothionein-like protein</td>
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**Secondary Metabolism**

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<td>248209_at</td>
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<td>Flavonol 3-O-glucosyltransferase-like protein</td>
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<td>247956_at</td>
<td>At5g56970</td>
<td>Cytokinin oxidase 3 (CKX3)</td>
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<td>264042_at</td>
<td>At2g03760</td>
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(Table continues on following page.)
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Table III. (Continued from previous page.)
accumulation arises through posttranscriptional regulation.

Based on the signal level from the microarray data, at least nine genes encoding AGP core polypeptides were actively expressed in our Arabidopsis culture cells (Table VI). Four AGP genes, AtAGP19, AtFLA4, AtFLA5, and AtFLA17, are not annotated in Affymetrix ATH1 array. The signal levels varied from low to high among the genes within each of the four types of AGPs (classical AGPs, AG-peptides, Lys-rich AGPs, and fasciclin-like AGPs), but the overall tendency was for the fasciclin-like AGPs to be expressed at lower levels than the others. Many of the highly induced AGP genes in our cell culture are predicted to have a GPI-anchor (Schultz et al., 2002). Three AGP genes (AtAGP18, AtAGP21, AtAGP22; all with predicted GPI anchors) were up-regulated at least 2-fold at 1 h of (β-D-Glc)₃ treatment. The up-regulation of an AGP gene at 1 h may imply that AGP is a component in the matrix deposits at the plasma membrane-cell wall interface. Alternatively, AGPs up-regulated at 1 h might be involved in transporting other Golgi-synthesized polymers to the cell wall, as suggested by Gibeaut and Carpita (1991).

Table III. (Continued from previous page.)

<table>
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<th>Gene Description</th>
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<tr>
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<td>Putative protein Pro-rich protein APG</td>
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<tr>
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<td>At5g03610</td>
<td>Putative protein Pro-rich protein APG</td>
<td>2.14</td>
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<td>Class 1 nonsymbiotic hemoglobin (AHB1)</td>
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<tr>
<td>266884_at</td>
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<td>Uclacyanin II (UCC II)</td>
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<tr>
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<td>Putative ferric-chelate reductase</td>
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<td>264506_at</td>
<td>At1g09560</td>
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</tr>
<tr>
<td>264365_s_at</td>
<td>At1g03220</td>
<td>Strong similarity to extracellular dermal glycoprotein (EDGP)</td>
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<td>At4g27450</td>
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<td>247307_at</td>
<td>At5g63860</td>
<td>UVB-resistance protein (UVR8)</td>
<td>2.14</td>
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<td>264505_at</td>
<td>At1g09380</td>
<td>Putative nodulin protein</td>
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<td>Germin-like oxalate oxidase</td>
<td>2.00</td>
</tr>
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<td>252338_at</td>
<td>At3g48890</td>
<td>Putative progesterone-binding protein homolog (ATMP2)</td>
<td>2.00</td>
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</table>
induced more than 2-fold at 10 h of \((\beta\text{-D-Glc})_3\) treatment, but \(AtAGP18\), \(AtAGP22\), \(AtFLA1\), and \(AtFLA9\) were down-regulated more than 2-fold at 10 h (Supplemental Table III).

The significant changes in cell wall-related genes tended to be up-regulations at 1 h and down-regulations at 10 h of \((\beta\text{-D-Glc})_3\) treatment. Most of the up-regulated genes had functions in cell wall modification, rather than in cell wall synthesis. Only a few genes with annotated functions in cell wall synthesis were significantly induced by \((\beta\text{-D-Glc})_3\) treatment. Several apparent glycosyltransferases of unknown substrate specificity were induced at 1 h (Table II). Two cellulose synthase-related genes were moderately up-regulated, \(At4g24000\) at 10 h (Table III), and another (\(At1g02730\); Supplemental Table III) was strongly down-regulated at 10 h. Because only a few of the enzymes involved in

Table IV. Comparison of Arabidopsis gene expression up-regulated by 50 \(\mu\text{M} (\beta\text{-D-Glc})_3\) or by wounding

Data on wound-induced genes taken directly from Cheong et al. (2002), where the analysis was performed using an 8,000 gene Affymetrix microarray. Blanks in the table indicate that the expression change was less than a 2-fold increase.

<table>
<thead>
<tr>
<th>AGI Locus</th>
<th>Gene Description</th>
<th>Fold Change</th>
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<tr>
<td></td>
<td>((\beta\text{-D-Glc})_3)-Induced Genes/1 h</td>
<td>((\beta\text{-D-Glc})_3)-Induced Genes/10 h</td>
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<td>At4g33920</td>
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<td>Extra-large G-protein-like</td>
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<td>At4g39890</td>
<td>Ras family GTP-binding protein</td>
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<td>SGPI monomeric G-protein</td>
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<td>TINY-like transcription factor</td>
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<td>DREB1C/ CBF2</td>
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<td>ZAT11</td>
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<td>MYB51</td>
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<tr>
<td>At4g25810</td>
<td>At-XTH23 (XTR6)</td>
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<td>At-XTH22 (TCH4)</td>
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<td>At-XTH17 (XTR1)</td>
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<td>UDP-Gal 4-epimerase-like (MUR4)</td>
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<tr>
<td>At2g43620</td>
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<tr>
<td>At2g40000</td>
<td>Nematode-resistance protein related</td>
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<tr>
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<td>Similar to senescence-associated protein 5</td>
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<tr>
<td>At2g38870</td>
<td>Protease inhibitor related</td>
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<td>Peroxidase ATP24a</td>
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<td>At5g47910</td>
<td>Respiratory burst oxidase protein D, RBOHD</td>
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<tr>
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<td>At4g20860</td>
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</table>


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Table V. The expression of callose synthases in Arabidopsis cell cultures

Entries in the columns labeled Signal indicate relative transcript accumulations detected across the same Affymetrix ATH1 microarray upon application of sample from cells treated with (β-D-Glc)₃ for the indicated time period. Entries in the columns labeled Fold Change reflect transcript abundances in (β-D-Glc)₃-treated cells relative to abundances in control cells. Entries in the Expression Change column (NC, no change; I, increase; D, decrease) were determined using default values in the Microarray Suite (MAS) 5.0 software (Affymetrix).

<table>
<thead>
<tr>
<th>Probe Set No.</th>
<th>AGI Locus</th>
<th>1 h (β-D-Glc)₃</th>
<th>10 h (β-D-Glc)₃</th>
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<td>At2g31960</td>
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<td>At5g13000</td>
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<td>264112_at</td>
<td>At2g13680</td>
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<td>At1g06490</td>
<td>208.7</td>
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<td>At3g14570</td>
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<td>At4g03550</td>
<td>1,231.3</td>
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<td>At4g04970</td>
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<td>1.07</td>
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<td>251499_at</td>
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<td>263891_at</td>
<td>At2g36850</td>
<td>1,602.2</td>
<td>1.00</td>
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</table>

Entries in the columns labeled Signal indicate relative transcript accumulations detected across the same Affymetrix ATH1 microarray upon application of sample from cells treated with (β-D-Glc)₃ for 1 h of (β-D-Glc)₃ and (β-D-Glc)₃ for 10 h of (β-D-Glc)₃.

cell wall synthesis have been identified to date, it is possible other cell wall synthesis genes were among the many genes of unknown function that changed expression in response to (β-D-Glc)₃.

Many Genes Involved in Transcriptional Control Were Up-Regulated by (β-D-Glc)₃ Treatment

Genes involved in transcriptional control were of particular interest relative to elucidating the regulation of the downstream effector genes that were induced or repressed by aggregating AGPs with (β-D-Glc)₃. Seven WRKY family transcription factor genes, including WRKY 8, 33, 40, 46, 53, 72, and 75 (Table II), were up-regulated more than 2-fold at 1 h of (β-D-Glc)₃ treatment, whereas two, WRKY 9 and 75 (Table III), were up-regulated more than 2-fold at 10 h. The WRKY family transcription factors contain a conserved WRKYGQK heptapeptide sequence followed by a zinc-finger motif (Eulgem et al., 2000) and are involved in plant defense response (Maleck et al., 2000; Yu et al., 2001; Dong et al., 2003), wound response, senescence (Hinderhofer and Zentgraf, 2001; Robatzek and Somssich, 2001), and morphogenesis (Johnson et al., 2002). Three of these genes, WRKY 33, 40, and 53, have been previously reported to be induced 30 min after wounding (Cheong et al., 2002), and this early induction correlates with their early induction by (β-D-Glc)₃ treatment (Table IV).

Several members of the ERF/AP2 family of transcription factors were induced by (β-D-Glc)₃. Among those, AtERF1, AtERF5, AtERF6, AtERF11, RAV2, DREBIC/CBF2, and TINY-like genes were also induced by wounding of Arabidopsis leaves (Table IV; Cheong et al., 2002). Treatment with (β-D-Glc)₃ for 1 h induced four AP2 domain-containing TINY-like genes (Table II). The semidominant tiny mutant shows increased expression of TINY protein that affects cell shape and expansion and results in a dwarf phenotype. The tiny mutants have shorter hypocotyl cells, more bulbous leaf epidermal cells, and larger diameter leaf mesophyll cells (Wilson et al., 1996). As mentioned above, (β-D-Glc)₃ induces root epidermal cell bulging (Willats and Knox, 1996; Ding and Zhu, 1997). Although these observations involved different tissues, the similarity in cell shape changes may suggest that TINY is involved in the root epidermal cell shape change triggered by (β-D-Glc)₃.

Several Cys₂/His₂-type zinc-finger transcription factors, AZF2, ZAT10/STZ, ZAT11, and ZAT12 (Lippuner et al., 1996; Meissner and Michael, 1997; Takatsuji, 1999; Sakamoto et al., 2000), were induced at 1 h of (β-D-Glc)₃ treatment (Table II) with AZF2 also being induced at 10 h of (β-D-Glc)₃ (Table III). Expressions of the ZAT10/STZ, ZAT11, and ZAT12 genes have previously been shown to be induced by wounding (Table IV; Cheong et al., 2002). It has been suggested that ZAT11 and ZAT10/STZ function as active repressors in transcriptional regulation mediated by the EAR motif L₁/DLN₁/L₂(Δ)F in their C-terminal region (Ohts et al., 2001). It is possible that some genes down-regulated by (β-D-Glc)₃ at 10 h may be the target genes regulated by these two transcription factors. In the microarray experiment with 1 h of (β-D-Glc)₃ treatment, the 128-fold increase in expression of the ZAT11 gene was the greatest induction observed in the entire array. Two points regarding this very high induction are noteworthy. First, in this same array experiment, the (β-D-Man)₃ control induced the expression of ZAT11 by 11.31-fold, certainly significant but much less than the 128-fold induction by (β-D-Glc)₃. Second, replicate experiments with different batches of cells and RNA all showed ZAT11 to be highly induced by (β-D-Glc)₃, but the magnitude of this
induction varied considerably (3.8–122; see below “Reliability of Microarray Data” and Table VII). One possible explanation for this wide variation may be that the expression of ZAT11 is rapid and transient, and different batches of cell culture may peak in ZAT11 expression at slightly different times of exposure to (β-D-Glc)₃.

### Table VI. The expression of AGPs in Arabidopsis cell cultures

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Probe Set No.</th>
<th>AGI Locus</th>
<th>Signal</th>
<th>Dc</th>
<th>Fold Change</th>
<th>Expression Change</th>
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</thead>
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<td>At2g22470</td>
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<td>At4g40090</td>
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<td>At5g10430</td>
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<td>I</td>
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<td>NC</td>
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<td>249375_at</td>
<td>At5g40730</td>
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<td>P</td>
<td>1.23</td>
<td>I</td>
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<tr>
<td>AGP17/ K</td>
<td>267260_at</td>
<td>At2g23130</td>
<td>125.6</td>
<td>M</td>
<td>1.15</td>
<td>NC</td>
</tr>
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<td>AGP18/ K²</td>
<td>253050_at</td>
<td>At4g37450</td>
<td>5,237.4</td>
<td>P</td>
<td>2.30</td>
<td>I</td>
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<td>FLA1/ F</td>
<td>248074_at</td>
<td>At5g55730</td>
<td>784.6</td>
<td>P</td>
<td>0.81</td>
<td>NC</td>
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<td>FLA2/ F</td>
<td>254785_at</td>
<td>At4g12730</td>
<td>1,148.3</td>
<td>P</td>
<td>1.32</td>
<td>I</td>
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<td>FLA3/ F</td>
<td>257932_at</td>
<td>At2g24450</td>
<td>41.9</td>
<td>A</td>
<td>3.25</td>
<td>NC</td>
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<td>FLA6/ F</td>
<td>263376_at</td>
<td>At2g20520</td>
<td>14.9</td>
<td>A</td>
<td>1.23</td>
<td>NC</td>
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<td>FLA7/ F</td>
<td>263628_at</td>
<td>At2g04780</td>
<td>1,495.8</td>
<td>P</td>
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<td>I</td>
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<td>251395_at</td>
<td>At2g45470</td>
<td>135.8</td>
<td>A</td>
<td>0.81</td>
<td>NC</td>
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<td>FLA9/ F</td>
<td>265066_at</td>
<td>At1g03870</td>
<td>219.6</td>
<td>A</td>
<td>2.14</td>
<td>NC</td>
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<td>FLA10/ F</td>
<td>251394_at</td>
<td>At3g60900</td>
<td>50.1</td>
<td>A</td>
<td>1.32</td>
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<td>FLA11/ F</td>
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<td>0.93</td>
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<td>FLA12/ F</td>
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<td>P</td>
<td>0.93</td>
<td>NC</td>
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<tr>
<td>FLA13/ F</td>
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<td>P</td>
<td>1.23</td>
<td>NC</td>
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<td>FLA14/ F</td>
<td>257691_at</td>
<td>At3g12660</td>
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<td>A</td>
<td>1.23</td>
<td>NC</td>
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<tr>
<td>FLA15/ F</td>
<td>256672_at</td>
<td>At3g52370</td>
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<td>A</td>
<td>1.07</td>
<td>NC</td>
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<td>FLA16/ F</td>
<td>263942_at</td>
<td>At2g35860</td>
<td>625.2</td>
<td>P</td>
<td>1.41</td>
<td>I</td>
</tr>
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<td>FLA18/ F</td>
<td>259072_at</td>
<td>At3g11700</td>
<td>2,698.6</td>
<td>P</td>
<td>1.62</td>
<td>I</td>
</tr>
<tr>
<td>FLA19/ F</td>
<td>262506_at</td>
<td>At1g15190</td>
<td>226.2</td>
<td>A</td>
<td>1.07</td>
<td>NC</td>
</tr>
<tr>
<td>FLA20/ F</td>
<td>249323_at</td>
<td>At5g40940</td>
<td>19.9</td>
<td>A</td>
<td>0.87</td>
<td>NC</td>
</tr>
<tr>
<td>FLA21/ F</td>
<td>250652_at</td>
<td>At5g06920</td>
<td>68</td>
<td>A</td>
<td>0.87</td>
<td>NC</td>
</tr>
</tbody>
</table>

indicates the more abundant AGPs in the cell culture.

Receptor-Like Protein Kinase and Other Protein Kinase Genes with Expression Altered by (β-D-Glc)₃ Treatment

Receptor-like protein kinases of various classes function in perception and transduction of extracellular signals into cellular responses. The lectin receptor protein kinase class is of potential interest because of the high carbohydrate content of AGPs. Lectin

Responses to Binding of Arabinogalactan Proteins

receptor protein kinases have a legume lectin-like extracellular domain, a transmembrane domain, and a Ser/Thr protein kinase domain. The extracellular lectin-like domain presumably can bind to complex glycans (Hervé et al., 1996, 1999), including perhaps a Ser/Thr protein kinase domain. The extracellular domain, a transmembrane domain, and a transmembrane domain. Finally, two of these genes were induced in the control cells.

Table VII. Comparison of expression changes detected by microarray analysis and by real-time PCR

<table>
<thead>
<tr>
<th>Gene Description</th>
<th>AGI Locus</th>
<th>Treatment</th>
<th>Microarray</th>
<th>Real-Time PCR</th>
<th>Replication 1</th>
<th>Replication 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZAT11</td>
<td>At2g37430</td>
<td>1 h</td>
<td>128</td>
<td>122.41 ± 8.20</td>
<td>11.35 ± 2.09</td>
<td>3.82 ± 0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 h</td>
<td>11.31</td>
<td>6.94 ± 2.66</td>
<td>1.37 ± 0.04</td>
<td>1.38 ± 0.22</td>
</tr>
<tr>
<td>AtMPK3</td>
<td>At3g45640</td>
<td>1 h</td>
<td>4.59</td>
<td>4.33 ± 0.03</td>
<td>1.35 ± 0.13</td>
<td>1.60 ± 0.24</td>
</tr>
<tr>
<td>TINY-like</td>
<td>At1g33760</td>
<td>1 h</td>
<td>9.19</td>
<td>4.61 ± 0.28</td>
<td>9.77 ± 0.97</td>
<td>4.29 ± 0.80</td>
</tr>
<tr>
<td>β-1,3-glucanase</td>
<td>At5g55180</td>
<td>10 h</td>
<td>2.64</td>
<td>3.40 ± 0.19</td>
<td>1.35 ± 0.55</td>
<td>1.62 ± 0.37</td>
</tr>
<tr>
<td>Callose synthase</td>
<td>At3g07160</td>
<td>1 h</td>
<td>1</td>
<td>1.22 ± 0.16</td>
<td>0.76 ± 0.08</td>
<td>1.01 ± 0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 h</td>
<td>0.79 ± 0.06</td>
<td>0.70 ± 0.05</td>
<td>0.77 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 h</td>
<td>1.32</td>
<td>1.42 ± 0.10</td>
<td>1.10 ± 0.09</td>
<td>1.32 ± 0.32</td>
</tr>
</tbody>
</table>

*Real-time PCR quantification of gene expression changes as determined by using the same RNA preparations as used in the microarray experiment.  
*Real-time PCR replications obtained using two different RNA preparations from two different batches of (β-d-Glc)$_3$-treated and control cells.

Disease Resistance-Related Genes with Expression Altered by (β-d-Glc)$_3$

Expression of more than 20 plant disease resistance genes (R genes) or genes structurally related to R genes were induced within 1 h of aggregating cell surface AGPs with (β-d-Glc)$_3$ (Table II). Their induction was characterized by early and transient, since very few of these genes were induced above 2-fold at 10 h (Table III). Plant R genes are induced in gene-for-gene interactions conferring resistance toward pathogens (Keen, 1990; Holt et al., 2000; Dangl and Jones, 2001). Some of them are induced during defense responses, presumably preparing the entire plant to resist further pathogen invasion (Schenk et al., 2003). Several other genes involved in disease defense responses were induced by (β-d-Glc)$_3$ treatment (Tables II and III). These genes encoded WRKY transcription factors (Eulgem et al., 2000), glutathione S-transferase, peroxidase, phenylalanine ammonia lyase, β-1,3-glucanase (Keen and Yoshikawa, 1983), AtMPK3 (Asai et al., 2002), NDR1-like proteins (Century et al., 1997), NPR1-like proteins (Glazebrook et al., 1996; Cao et al., 1997), and EDs5 (Rogers and Ausubel, 1997). The induction of these defense genes and the formation of callose-containing matrix deposits between the plasma membrane and cell wall (Fig. 2) may suggest that (β-d-Glc)$_3$ treatment induced defense-like responses. It has been shown that some genes induced by wounding are involved in pathogen response (Cheong et al., 2002), so that defense genes may be induced by (β-d-Glc)$_3$ via the wound-response pathway. We do not know if (β-d-Glc)$_3$-mediated

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induction of plant defense genes effectively enhances plant resistance toward pathogens.

Many Genes Involved in Wound Response Were Up-Regulated by (β-d-Glc)_3 Treatment

The earlier work of Cheong et al. (2002), where the 8,000 gene Affymetrix microarray was used to investigate transcriptional profiling of the wound response, provided an excellent opportunity for a large-scale comparison between the responses to (β-d-Glc)_3 treatment and to wounding. Even with the different array used by Cheong et al., the comparison showed that more than 40 genes exhibited commonality of expression (Table IV). Although the early (1 h versus 30 min) and late (10 h versus 6 h) time points were not precisely matched between the two studies and the tissue types receiving the treatment were different, it is apparent that the similarity of expressions between the two experiments extended to both time course and magnitude for many genes. The commonality between (β-d-Glc)_3 treatment and wounding was particularly evident for transcription factors, which account for nearly one-half of the entries in Table IV. Genes encoding signal transduction pathway components (receptor-like kinases and G-proteins) and cell wall-related proteins (glycosyltransferase, cellulose synthase, and hydrolases) also showed some expression similarities between the two treatments.

Although jasmonic acid signaling is involved in plant wound and pathogen responses (Glazebrook, 2001; León et al., 2001), we did not observe (β-d-Glc)_3 induction of PDF1.2, JR1, JR2, or some other genes typically induced by jasmonic acid. Rojo et al. (1998) could not detect jasmonic acid-induced activation of JR genes in Arabidopsis cell cultures and suggested that other hormones in the cell culture medium may interfere with jasmonic acid-mediated signaling.

Reliability of Microarray Data

To test the reliability of the microarray data, the mRNA abundances for several genes of interest were determined by an alternate method, real-time PCR analysis. When applied to the same RNA preparations, the microarray analysis and the real-time PCR analysis yielded very similar results (Table VII), indicating the reliability of the microarray analysis. To assess the variation in the sample preparation, transcript abundances from two additional separate batches of cells were analyzed using real-time PCR. Although considerable variation was observed among the expression fold changes for any particular gene in the three real-time PCR experiments (Table VII), the ordering of fold changes within the set of genes was quite similar among the three experiments. As an extreme example, the ZAT11 gene had one of the highest fold expression changes at 1 h in each experiment, but these ranged from 3.82-fold to 128-fold among the three experiments. Comparison of Tables II and III shows that the expression of many genes is highly transient, so it is likely that substantial variation in 1 h expression levels among repeated experiments can arise if the cells respond slightly faster or slower in the different experiments.

CONCLUSION

Although some progress has been recently reported (Shi et al., 2003; Van Hengel and Roberts, 2003), the mutational approach to investigating AGP functions has typically been hampered by absence of detectable phenotypes. Because the Arabidopsis genome contains approximately 47 genes encoding AGP core polypeptides (Schultz et al., 2002), considerable possibility exists for functional redundancy (Johnson et al., 2003). An alternative approach to studying AGP function, and the approach taken in this work, involves use of (β-d-Glc)_3, a synthetic chemical that specifically binds, precipitates, and presumably activates a wide range of AGPs (Nothnagel, 1997), including those containing a fasciclin-like domain (Johnson et al., 2003). Treatment with (β-d-Glc)_3 generally produces a profound phenotype. For example, 50 μM (β-d-Glc)_3 abruptly and completely stops the growth of plant cell cultures, and we know of no plant species that does not show this effect. A disadvantage of the broad AGP specificity of (β-d-Glc)_3 is that observed effects cannot be attributed to any specific AGP.

This study shows that Arabidopsis cell cultures are induced to increase accumulation of mRNAs from a wide variety of genes at 1 and 10 h of 50 μM (β-d-Glc)_3 treatment, and to decrease accumulation of many other mRNAs at 10 h of treatment. When carefully analyzed and coupled with observations by microscopy, the diverse accumulation of mRNAs clarifies to reveal some trends. Foremost among these trends is similarity to wound-like responses, including cell wall thickening, callose synthesis (Fig. 2), and induction of genes encoding certain transcription factors, cell wall-related proteins, and signal transduction components (Table IV).

These similarities to wound response and possibly pathogen response lead to the question of how aggregation of AGPs might mimic some aspect of the cellular or molecular processes that occur during wounding or pathogen attack. The simplest hypothesis might be that (β-d-Glc)_3-induced aggregation of plasma membrane AGPs generates physical stresses that directly damage the membrane, tearing it open, much as insect feeding or other wounding opens the membrane. Although the death of Arabidopsis cells caused by (β-d-Glc)_3 treatment might seem consistent with this hypothesis of membrane tearing, membrane tearing should produce very rapid cell death. Instead, (β-d-Glc)_3 treatment results in a very gradual onset of cell death (Fig. 1). Furthermore, earlier work (Serpe and Nothnagel, 1994) showed that rose cells suffer...
germinated on agar-solidified medium. The resulting callus was subcultured


mutants for altered responses to (β-D-Glc)₃ treatment. We are now obtaining Arabidopsis cells treated with (β-D-Glc)₃ or near these microdomains. These AGP-associated proteins, rather than the AGPs themselves, might be the effective signal transduction pathway components.

Irrespective of whether AGPs directly or indirectly participate in a signal transduction pathway, an important remaining issue is the possible biological ligands for AGPs during a wound response. Little is known about ligands for AGPs in molecular-level interactions in any context, with the sparse data pointing to possible bindings of AGPs with pectins or flavonol β-glycosides (Nothnagel, 1997; Muñiz and Riezman, 2000). At least one report suggests that such microdomains are also present in plant cells (Peskano et al., 2000). If microdomains are present in plant cells, then the (β-D-Glc)₃-AGP aggregates formed on plasma membranes may trap other nearby membrane proteins in these microdomains. These AGP-associated proteins, rather than the AGPs themselves, might be the effective signal transduction pathway components.

The method of Verwoerd et al. (1989) was used to extract RNA from control and treatment Arabidopsis cell cultures. Stocks of total RNAs were prepared to a final concentration of 1 µg µL⁻¹ for subsequent microarray analysis. The procedures for the microarray analysis followed the recommendations of the manufacturer (Affymetrix GeneChip Expression Analysis Technical Manual, Affymetrix, Santa Clara, CA) and were largely performed at the UCI DNA Microarray Facility (University of California, Irvine). Quality of total RNA samples was assessed by electrophoretic separation of a small aliquot of each sample on a RNA lab-on-a-chip (Caliper Technologies, Mountain View, CA) with subsequent analysis on an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA). Double-stranded cDNAs were generated using the SuperScript Double-Stranded cDNA synthesis kit (Invitrogen, Carlsbad, CA) and T7-dT₇ oligonucleotide primers, which contained a sequence recognized by T7 RNA polymerase. Generation of biotin-tagged cRNA from an in vitro transcription reaction was accomplished using a BioArray HighYield RNA transcript labeling kit (Enzo Diagnostics, New York). Labeled cRNA (15 µg) was fragmented to the size of 35 to 200 bases, and then 10 µg of the fragments were hybridized to the Affymetrix Arabidopsis ATH1 whole genome array for 16 h at 45°C with rotation. After hybridization the arrays were washed and stained with streptavidin-phycocerythrin on an Affymetrix Fluidics Station 4000 and then scanned with a GeneArray Scanner (Hewlett-Packard, Palo Alto, CA). The results were quantified and analyzed using MicroArray Suite 5.0 software (Affymetrix) using default values (scaling, target signal = 500; normalization, all probe sets; parameters, all set at default signal).

**Quantitative PCR**

Labeling for quantitative PCR was performed with the Brilliant SYBR Green QPCR core reagent kit (Stratagene, La Jolla, CA). Primers for each gene were designed using Primer Express software (Applied Biosystems, Foster City, CA). Genes and their primers were: AtMAPK3, 5'-ccacagagttctggccacacc, 5'-gctaacaggtgtaacgccga; ZAT11, 5'-gagatattcttgtaacagcc, 5'-ttcacattttc- gcaaacag; TINY-like, 5'-ccacaggtcgagctgacg, 5'-tgccacaagtgcaggtctg; β-1,3-glucanase, 5'-ttgaccagactcgagagctgg; 5'-gacagagttgcttggcacacc, 5'-attcaggtcgggaagggac; callose synthase (At1g07160), 5'-ttgaccagactcgagagctgg; 5'-gacagagttgcttggcacacc, 5'-attcaggtcgggaagggac; callose synthase (At4g03550), 5'-attcaggtcgggaagggac; 5'-catcaactaactgtagtagtag; and actin 2/7, 5'-ctcatctttcgttcctggag; 5'-acacacaaattacgttgcc; callose synthase (At1g07160), 5'-ttgaccagactcgagagctgg; 5'-gacagagttgcttggcacacc, 5'-attcaggtcgggaagggac; callose synthase (At4g03550), 5'-attcaggtcgggaagggac; 5'-catcaactaactgtagtagtag; and actin 2/7, 5'-ctcatctttcgttcctggag; 5'-acacacaaattacgttgcc. Quantitative PCR was performed using ABI PRISM7700 Sequence Detection System (Applied Biosystems). Relative quantitation was done by the standard curve method with standard curves generated for both the target genes and the actin standard.

**ACKNOWLEDGMENTS**

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**MATERIAL AND METHODS**

**Arabidopsis Cell Culture and Treatment**

Arabidopsis ecotype Columbia cell cultures were initiated from seeds germinated on agar-solidified medium. The resulting callus was subcultured weekly in liquid B5 medium (Gamborg et al., 1968) in the dark. Experimental treatments were applied at the time of subculture and involved transferring 4 mL of packed cell volume to 40 mL of B5 medium or B5 medium supplemented with 50 µM (β-D-Glc), or 50 µM (β-D-Man).

**Histochemistry**

Callose in Arabidopsis cells was visualized by fluorescence staining with Aniline Blue, or more accurately with Sirofluor, a callose-staining fluochrome found as a minor component in commercial Aniline Blue preparations (Stone et al., 1984). Aniline Blue was applied to the Arabidopsis cells at 1 mg mL⁻¹ in 0.07 M potassium phosphate buffer, pH 8.5. Viability of Arabidopsis cells was assessed with the vital fluorescent stain fluorescein diacetate (Huang et al., 1986) applied at 0.05 mg mL⁻¹ (1:100 dilution from 5 mg mL⁻¹ stock in acetone) in B5 medium.

**Microarray Experiments**

The method of Schulte et al. (1998, 2002). In animal cells, many GPI-anchored proteins are localized in plasma membrane microdomains where they associate with specific groups of transmembrane proteins (Peles et al., 1997; Muñiz and Riezman, 2000). At least one report suggests that such microdomains are also present in plant cells (Peskano et al., 2000). If microdomains are present in plant cells, then the (β-D-Glc)₃-AGP aggregates formed on plasma membranes may trap other nearby membrane proteins in these microdomains. These AGP-associated proteins, rather than the AGPs themselves, might be the effective signal transduction pathway components.

Irrespective of whether AGPs directly or indirectly participate in a signal transduction pathway, an important remaining issue is the possible biological ligands for AGPs during a wound response. Little is known about ligands for AGPs in molecular-level interactions in any context, with the sparse data pointing to possible bindings of AGPs with pectins or flavonol β-glycosides (Nothnagel, 1997). Yariv phenylglycosides self associate in aqueous solutions to form complexes of 10 to 50 molecules, and it has also been speculated that the arrangement of sugars in these complexes resembles callose or some other naturally occurring macromolecule with which AGPs interact in plant cells (Nothnagel, 1997).

The gene expression profile data obtained in this study can be utilized in further studies aimed toward improving our understanding of AGPs. For example, we have selected a subset of the genes whose expression was found to be significantly altered by (β-D-Glc)₃ treatment in this study. We are now obtaining Arabidopsis mutants carrying T-DNA inserts in or near these genes and are screening seedlings of these mutants for altered responses to (β-D-Glc)₃ treatment. We hope this screen will help identify genes that are involved in AGP-mediated signaling.
LITERATURE CITED


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