Binding of Arabinogalactan Proteins by Yariv Phenylglycoside Triggers Wound-Like Responses in Arabidopsis Cell Cultures[1][w]

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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 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; Gibeaut 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 $(\beta\text{-D-Glc})_3$ are synthetic probes that bind and aggregate AGPs. The $(\beta\text{-D-Man})_3$ Yariv phenylglycoside differs from the $(\beta\text{-D-Glc})_3$ Yariv phenylglycoside only by isomerization of the hydroxyl group at carbon atom 2 of the sugar. Although $(\beta\text{-D-Glc})_3$ and $(\beta\text{-D-Man})_3$ are extremely close structural analogs, $(\beta\text{-D-Glc})_3$ binds AGPs but $(\beta\text{-D-Man})_3$ does not, making the latter an excellent control (Yariv et al., 1967; Nothnagel, 1997).

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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-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-Glc$)_3$ also induces phenovariation in *Streptocarpus prolixus* (Rauh and Basile, 2003).

To elucidate the effects triggered by $(\beta$-D-Glc$)_3$ and to further understand AGP functions, we used Arabidopsis cell cultures treated with $(\beta$-D-Glc$)_3$. When applied to Arabidopsis seedlings, $(\beta$-D-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-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-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-Glc$)_3$ Yariv Phenylglycoside

The viability of Arabidopsis cell cultures decreased to 50% within approximately 36 h after start of exposure to 50 μM $(\beta$-D-Glc$)_3$ (Fig. 1). Similar exposure to 50 μM $(\beta$-D-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-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-Glc$)_3$ treatment and increased up to at least 36 h (Fig. 2). Treatment with 50 μM $(\beta$-D-Man$)_3$ did not induce callose deposition (data not shown). Callose, a $(1\rightarrow3)$-β-D-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).

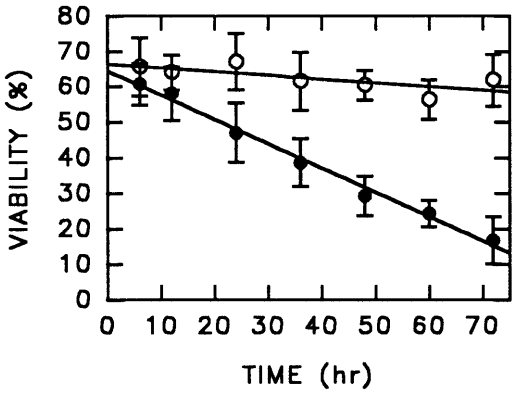

Figure 1. Effect of $(\beta$-D-Glc$)_3$ on viability of Arabidopsis cells, as monitored by fluorescein diacetate staining. At time 0 h, suspension culture cells were transferred to either 50 μM $(\beta$-D-Glc$)_3$ in fresh B5 medium (●) or fresh B5 medium alone as the control (○). Experiments were repeated at least four times. Bars indicate SD.

The $(\beta$-D-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-Glc$)_3$ binding of cell surface AGPs triggers wound-like responses.

Overview of Gene Expression Changes Resulting from $(\beta$-D-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-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-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-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-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

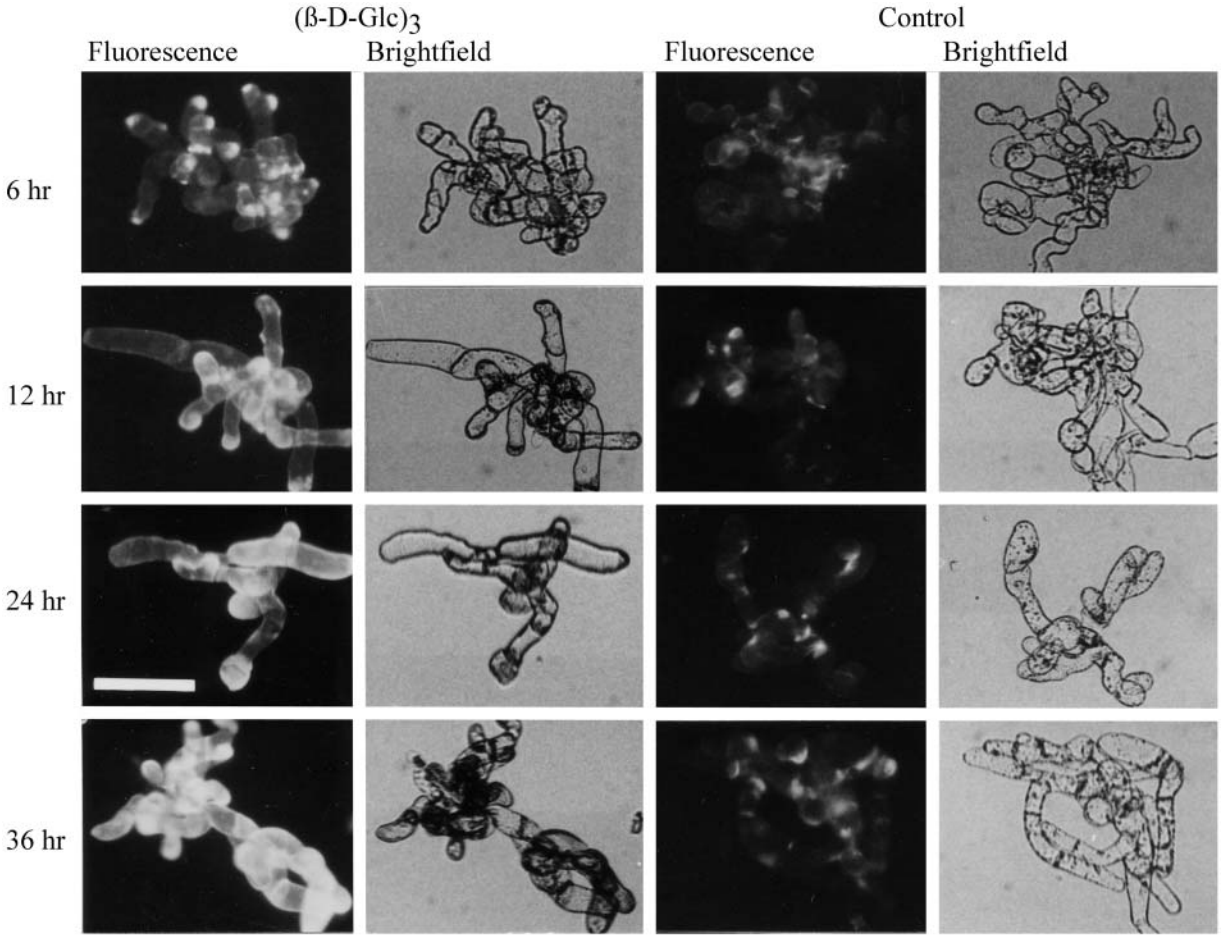

Figure 2. Effect of $(\beta\text{-D-Glc})_3$ on callose deposition in Arabidopsis cells, as visualized by histochemical fluorescence staining with Aniline Blue. At time 0 h, suspension culture cells were transferred to either 50 μM $(\beta\text{-D-Glc})_3$ in fresh B5 medium or fresh B5 medium alone as the control. Bar represents 150 μm.

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 on-going 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 $(\beta\text{-D-Glc})_3$-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 $(\beta\text{-D-Glc})_3$ 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 $(\beta\text{-D-Glc})_3$-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 $(\beta\text{-D-Man})_3$ treatment. While 50 μM $(\beta\text{-D-Glc})_3$ induced 410 genes within 1 h of treatment, 50 μM $(\beta\text{-D-Man})_3$ 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 $(\beta\text{-D-Man})_3$, 20 were also changed in expression by $(\beta\text{-D-Glc})_3$. 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 $(\beta\text{-D-Glc})_3$ Treatment

Arabidopsis cell death triggered by $(\beta\text{-D-Glc})_3$ 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 $(\beta\text{-D-Glc})_3$ treatment (Tables II and III). These genes included those with sequence similarities to senescence associated genes *DSA5* (At2g23810,

Table I. *Summary of genes with mRNA accumulation increased or decreased at least 2-fold at 1 h or 10 h of 50 μM (β-D-Glc)$_3$ treatment compared to B5 medium control*

Nineteen additional genes had mRNA accumulation increased or decreased at least 2-fold at 1 h of both 50 μM (β-D-Glc)$_3$ treatment and 50 μM (β-D-Man)$_3$ treatment. These 19 genes are footnoted in Supplemental Table V and, with the exception of *ZAT11*, are excluded from all other tables. Treatment was initiated at the time of transfer to fresh medium. Based principally upon the annotations given at The Arabidopsis Information Resource (TAIR) Web site (www.arabidopsis.org), genes were categorized as either known genes or unknown genes. The resulting category of known genes was further divided to genes with classified function or genes with unclassified function, based principally upon the annotations given at the Munich Information Center for Protein Sequencing (MIPS) Web site (http://mips.gsf.de).

Gene Category	Number of Genes in Category			
	Increased mRNA		Decreased mRNA	
	1 h	10 h	1 h	10 h
Unknown genes	149	97	29	137
Known genes with classified function	235	177	28	199
Known genes with unclassified function	27	31	6	33

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 (*YLS9*; At2g35980; Pontier et al., 1999; Yoshida et al., 2001). Also induced by (β-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 (β-D-Glc)$_3$ Treatment

Treatment with (β-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 (β-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 AGP-like gene (Shi et al., 2003). Cell bulging was not apparent in our (β-D-Glc)$_3$-treated Arabidopsis culture cells (Fig. 2), and we have previously shown that cell volume does not appreciably change in (β-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 (β-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 (β-D-Glc)$_3$ treatment (Table II). No XTH genes were up-regulated at 10 h. Three of the induced XTH genes, *At-XTH17* (*XTR1*), *At-XTH22* (*TCH4*), and *AT-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, *AtEXP12*, *EXPL2*, and *EXPL3*, were induced slightly more than 2-fold at 1 h of (β-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 (β-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 β-1,3-glucanase genes were induced at either 1 h or 10 h of (β-D-Glc)$_3$ treatment. The 22-fold increase in expression of 1 β-1,3-glucanase gene (At3g04010) was the strongest induction observed on the entire microarray at 10 h (Table III). Many β-1,3-glucanases are involved in plant defense (Keen and Yoshikawa, 1983; Sela-Buurlage et al., 1993) or development (Bucciaglia and Smith, 1994; Delp and Palva, 1999; Buchner et al., 2002), and some β-1,3-glucanases are induced by wounding or hormone

Table II. *Known genes up-regulated at least 2-fold at 1 h of 50 μM (β-D-Glc)$_3$ treatment*

See Table I caption for other details.

Probe Set No.	AGI Locus	Gene Description	Fold Increase
		Cell Rescue, Defense, Cell Death, and Aging	
267293_at	At2g23810	Similar to senescence-associated protein 5	2.30
255479_at	At4g02380	Senescence-associated gene 21 (SAG21)	2.46
252921_at	At4g39030	Enhanced disease susceptibility 5 (EDS5)	2.00
255504_at	At4g02200	Drought-induced protein-related	2.30
252988_at	At4g38410	Similar to dehydrin ERD10	2.00
252102_at	At3g50970	Dehydrin Xero2	2.46
252906_at	At4g39640	Putative gamma-glutamyltransferase	2.64
263948_at	At2g35980	YLS 9, hin1 homolog	6.06
250676_at	At5g06320	NDR1/HIN1-like protein 3 (NHL3)	2.64
259071_at	At3g11650	NDR1/HIN1-like protein 2 (NHL2)	2.46
254014_at	At4g26120	NPR1 like protein	2.30
265008_at	At1g61560	MLO6	2.30
267357_at	At2g40000	Nematode-resistance protein-related	4.59
251774_at	At3g55840	Nematode-resistance protein-related	3.25
247848_at	At5g58120	Similar to disease resistance protein RPP1-WsA	2.00
252648_at	At3g44630	Disease resistance protein RPP1-WsB-like	2.00
258577_at	At3g04220	Similar to disease resistance protein RPP1-WsC	2.64
254905_at	At4g11170	TIR-NBS-LRR class putative disease resistance protein	3.48
245654_at	At1g56540	TIR-NBS-LRR class disease resistance protein	2.64
249264_s_at	At5g41740	TIR-NBS-LRR class disease resistance protein	2.30
249029_at	At5g44870	TIR-NBS-LRR class disease resistance protein	2.14
249903_at	At5g22690	TIR-NBS-LRR class disease resistance protein	2.00
252126_at	At3g50950	CC-NBS-LRR class disease resistance protein	2.30
265597_at	At2g20145	TIR-class disease resistance protein	4.59
265136_at	At1g51280	TIR-class disease resistance protein	2.46
249032_at	At5g44910	TIR-class disease resistance protein	2.00
265723_at	At2g32140	TIR class disease resistance protein	4.29
262381_at	At1g72900	TIR-NBS class similar to virus resistance protein	3.25
262382_at	At1g72920	TIR-NBS class similar to virus resistance protein	2.46
256526_at	At1g66090	TIR-NBS class disease resistance protein	5.66
245033_at	At2g26380	LRR disease resistance protein-related	2.46
259805_at	At1g47890	LRR disease resistance protein	2.46
267411_at	At2g34930	LRR disease resistance protein	3.48
246916_at	At5g25910	LRR disease resistance protein	2.64
260406_at	At1g69920	Similar to glutathione transferase	3.73
248719_at	At5g47910	Respiratory burst oxidase protein D (RBOHD)	2.64
253496_at	At4g31870	Glutathione peroxidase	2.00
261474_at	At1g14540	Putative anionic peroxidase	18.38
261475_at	At1g14550	Putative anionic peroxidase	2.00
250702_at	At5g06730	Peroxidase	2.30
250646_at	At5g06720	Peroxidase	2.14
249459_at	At5g39580	Peroxidase ATP24a	4.59
		Cell Wall	
258764_at	At3g10720	Putative pectinesterase	5.28
245151_at	At2g47550	Putative pectinesterase	3.48
252989_at	At4g38420	Putative pectinesterase	3.03
255524_at	At4g02330	Similar to pectinesterase	3.73
259033_at	At3g09410	Putative pectinacetylesterase	3.03
255175_at	At4g07960	Similar to cellulose synthase (AtCSLC12)	2.00
257950_at	At3g21780	Putative UDP-Glc glucosyltransferase	2.14
252179_at	At3g50760	Glycosyltransferase	2.14
265499_at	At2g15480	Glucosyltransferase-related	2.14
265501_at	At2g15490	Glucosyltransferase-related	2.00
265199_s_at	At2g36770	Glycosyltransferase	4.59
251971_at	At3g53160	UDP-glycosyltransferase	2.00
264857_at	At1g24170	Glycosyltransferase	2.14
257203_at	At3g23730	At-XTH16	2.64
264157_at	At1g65310	At-XTH17 (XTR1)	5.66

(*Table continues on following page.*)

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

Probe Set No.	AGI Locus	Gene Description	Fold Increase
253628_at	At4g30280	At-XTH18	2.64
247925_at	At5g57560	At-XTH22 (TCH4)	2.14
254042_at	At4g25810	At-XTH23 (XTR6)	3.03
253666_at	At4g30270	At-XTH-24 (meri5B)	2.83
245794_at	At1g32170	At-XTH30 (XTR4)	3.25
258388_at	At3g15370	AtEXP12	2.30
252997_at	At4g38400	EXPL2	2.30
252557_at	At3g45960	EXPL3	2.14
253050_at	At4g37450	AGP18	2.30
259664_at	At1g55330	AGP21	2.00
248252_at	At5g53250	AGP22	2.83
254770_at	At4g13340	Leu-rich repeat extensin	3.03
265114_at	At1g62440	Leu-rich repeat/extensin2 (LRX2)	2.14
260556_at	At2g43620	Putative endochitinase	2.30
259443_at	At1g02360	Putative chitinase	2.14
265648_at	At2g27500	Putative beta-1,3-glucanase	2.00
264280_at	At1g61820	Putative beta-glucosidase	3.03
251456_at	At3g60120	Beta-glucosidase-like	2.00
255756_at	At1g19940	Putative endo-beta-1,4-D-glucanase	3.03
258631_at	At3g07970	Putative polygalacturonase	3.03
		Cytoskeleton	
252873_at	At4g40020	Putative protein myosin heavy chain	2.14
256275_at	At3g12110	Actin 11 (ACT11)	2.14
		Plant Development	
250024_at	At5g18270	NAM-like protein	2.00
		Cellular Communication/Signal Transduction	
253103_at	At4g36110	Putative auxin-induced protein	2.64
250279_at	At5g13200	ABA-responsive protein-like	2.00
252592_at	At3g45640	AtMPK3	4.59
254924_at	At4g11330	AtMPK5	2.00
259428_at	At1g01560	AtMPK11	2.00
253937_at	At4g26890	MAPKKK16	2.30
248090_at	At5g55090	MAPKKK15	2.46
247137_at	At5g66210	Calcium dependent protein kinase 28 (CPK28)	2.00
251636_at	At3g57530	Calcium dependent protein kinase 32 (CPK32)	2.00
262228_at	At1g68690	Similar to protein kinase 1	2.30
254605_at	At4g18950	Protein kinase 6	2.83
251494_at	At3g59350	Similar to Pto kinase interactor 1	2.00
257840_at	At3g25250	Putative protein kinase	5.28
266037_at	At2g05940	Putative protein kinase	2.83
266196_at	At2g39110	Putative protein kinase	2.83
267289_at	At2g23770	Putative protein kinase	2.83
249705_at	At5g35580	Putative protein kinase	2.64
264232_at	At1g67470	Putative protein kinase	2.30
246608_at	At5g35380	Putative protein kinase	2.83
253747_at	At4g29050	lecRK1-like	8.57
251054_at	At5g01540	Similar to receptor lectin kinase 3	2.00
264718_at	At1g70130	Similar to receptor lectin kinase 3	4.59
261394_at	At1g79680	WAKL10	2.14
257478_at	At1g16130	WAKL2	2.00
254241_at	At4g23190	RLK3	2.14
254898_at	At4g11480	Similar to RLK3	5.28
254256_at	At4g23180	RLK4	2.14
246858_at	At5g25930	RLK5 like	2.46
260975_at	At1g53430	Putative receptor Ser/Thr protein kinase	2.00
247145_at	At5g65600	Receptor-like protein kinase	3.48
259213_at	At3g09010	Putative receptor Ser/Thr protein kinase	2.14
247617_at	At5g60270	Receptor-like protein kinase	2.00
253323_at	At4g33920	Putative protein phosphatase	2.00

(*Table continues on following page.*)

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

Probe Set No.	AGI Locus	Gene Description	Fold Increase
259859_at	At1g68410	Similar to protein phosphatase-2C	2.00
249197_at	At5g42380	Calmodulin-related protein	16.00
246821_at	At5g26920	Calmodulin-binding protein	3.73
252417_at	At3g47480	Calcium-binding EF-hand protein	2.14
256755_at	At3g25600	Similar to calmodulin	2.00
260068_at	At1g73805	Putative calmodulin-binding protein	3.48
249417_at	At5g39670	Calcium-binding EF-hand protein	2.83
254487_at	At4g20780	Calcium-binding protein	2.64
267083_at	At2g41100	Calmodulin-like 5 (AtCAL5) (TCH3)	2.83
260881_at	At1g21550	Calcium-binding protein	2.30
260135_at	At1g66400	Calmodulin-related protein	3.03
260046_at	At1g73800	Calmodulin-binding protein	2.46
255599_at	At4g01010	Cyclic nucleotide gated channel (CNGC13)	2.30
252825_at	At4g39890	Ras family GTP-binding protein	2.00
253257_at	At4g34390	Extra-large G-protein-like	2.00
		Cell Growth, Cell Division, and DNA Synthesis	
267393_at	At2g44500	axi 1 (auxin independent growth)-related	3.73
253271_s_at	At4g34210	SKP1-like 11	2.14
		Metabolism	
252070_at	At3g51680	Short-chain alcohol dehydrogenase	3.73
267169_at	At2g37540	Short-chain dehydrogenase/reductase family protein	3.03
256319_at	At1g35910	Similar to trehalose-6-phosphate phosphatase	3.48
250467_at	At5g10100	Similar to trehalose-6-phosphate phosphatase	3.03
265841_at	At2g35710	Glycogenin glucosyltransferase (glycogenin)-related	2.46
265221_s_at	At2g02010	Glutamate decarboxylase	4.00
252652_at	At3g44720	Putative prephenate dehydratase	2.30
249910_at	At5g22630	Chorismate mutase/prephenate dehydratase-like protein	3.48
265725_at	At2g32030	Putative Ala acetyl transferase	18.38
254158_at	At4g24380	Putative dihydrofolate reductase	2.64
261933_at	At1g22410	Putative 2-dehydro-3-deoxyphosphoheptonate aldolase	2.14
254707_at	At4g18010	Inositol polyphosphate 5-phosphatase II (IP5PII)	2.46
252976_s_at	At4g38550	Phospholipase-like protein	2.14
254847_at	At4g11850	Phospholipase D-gamma (PLDGAMMA1)	2.14
245447_at	At4g16820	Triacylglycerol lipase-like protein	2.00
267318_at	At2g34770	Fatty acid hydroxylase (FAH1)	2.14
263198_at	At1g53990	GDSL-motif lipase/hydrolase protein	2.14
249333_at	At5g40990	GDSL-motif lipase/hydrolase-like protein	2.46
260399_at	At1g72520	Putative lipoxygenase	6.50
261037_at	At1g17420	Putative lipoxygenase	3.48
262745_at	At1g28600	Putative lipase	2.00
265737_at	At2g01180	Phosphatidic acid phosphatase (AtPAP1)	3.03
252870_at	At4g39940	Adenosine-5-phosphosulfate-kinase (AKN2)	2.00
251028_at	At5g02230	Haloacid dehalogenase-like	2.64
		Secondary Metabolism	
256922_at	At3g19010	Contains similarity to flavonol synthase	2.00
254926_at	At4g11280	ACC synthase (AtACS-6)	2.14
263845_at	At2g37040	Phenylalanine ammonia lyase (PAL1)	3.48
251984_at	At3g53260	Phenylalanine ammonia-lyase (PAL2)	5.66
249188_at	At5g42830	N-hydroxycinnamoyl benzoyltransferase-like protein	5.28
251124_s_at	At5g01040	Laccase-like protein	3.25
261449_at	At1g21120	Putative *O*-methyltransferase 1	2.64
261453_at	At1g21130	Putative *O*-methyltransferase 1	3.73
261459_at	At1g21100	Putative *O*-methyltransferase 1	2.46
261450_s_at	At1g21110	Putative *O*-methyltransferase 1	2.14
261899_at	At1g80820	Cinnamoyl CoA reductase isoform 2 (CCR2)	2.14
262744_at	At1g28680	Similar to *N*-hydroxycinnamoyl/benzoyltransferase	2.14
254447_at	At4g20860	Similar to reticulin oxidase	2.30
261021_at	At1g26380	Similar to reticulin oxidase	9.19
261005_at	At1g26420	Similar to reticulin oxidase	4.59

(*Table continues on following page.*)

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

Probe Set No.	AGI Locus	Gene Description	Fold Increase
		Cytochrome P450	
254562_at	At4g19230	CYP707A1	2.30
247949_at	At5g57220	CYP81F2	5.66
248964_at	At5g45340	CYP707A3	3.73
248358_at	At5g52400	CYP715A1	3.25
253503_at	At4g31950	CYP82C3	2.30
252827_at	At4g39950	CYP79B2	2.14
		Protein Destination	
266168_at	At2g38870	Protease inhibitor-related	5.66
250944_at	At5g03380	Farnesylated protein-like	2.30
264866_at	At1g24140	Metallo proteinase-related	4.59
245738_at	At1g44130	Putative nucellin	2.64
264716_at	At1g70170	Matrix metalloproteinase (MMP)	2.46
249626_at	At5g37540	Putative protein nucleoid DNA-binding protein cnd41	2.14
263614_at	At2g25240	Ser protease inhibitor (putative serpin)	2.00
245034_at	At2g26390	Ser protease inhibitor (putative serpin)	2.00
		Transcription	
261984_at	At1g33760	TINY-like protein	9.19
260856_at	At1g21910	TINY-like protein	2.46
264202_at	At1g22810	TINY-like protein	2.46
261327_at	At1g44830	TINY-like protein	2.30
254075_at	At4g25470	DREB1C/CBF2	2.30
252214_at	At3g50260	EREBP-3 homolog, *Stylosanthes hamata*	4.92
247543_at	At5g61600	Putative EREBP-4	2.64
248799_at	At5g47230	AtERF5	2.00
245250_at	At4g17490	AtERF6	4.92
261470_at	At1g28370	Similar to AtERF11	2.30
267451_at	At2g33710	Similar to RAP2.6	2.14
260037_at	At1g68840	RAV2	2.64
248896_at	At5g46350	WRKY8	2.14
267028_at	At2g38470	WRKY33	2.64
261892_at	At1g80840	WRKY40	3.25
263783_at	At2g46400	WRKY46	2.46
254231_at	At4g23810	WRKY53	2.30
250153_at	At5g15130	WRKY72	2.14
245976_at	At5g13080	WRKY75	2.64
251745_at	At3g55980	Zinc finger transcription factor (PEI1)	4.92
261648_at	At1g27730	ZAT10/STZ	3.25
266010_at	At2g37430	ZAT11	128.00
247655_at	At5g59820	ZAT12	4.92
257022_at	At3g19580	AZF2	2.14
251861_at	At3g54810	GATA transcription factor 3	2.46
256576_at	At3g28210	Zinc finger protein (PMZ)	2.14
254919_at	At4g11360	RING-H2 finger protein (RHA1B)	3.25
254922_at	At4g11370	RING-H2 finger protein (RHA1A)	2.46
246777_at	At5g27420	RING-H2 zinc finger protein (ATL6)	2.46
257919_at	At3g23250	MYB15	3.03
255753_at	At1g18570	MYB51	3.03
246253_at	At4g37260	MYB73	2.14
252193_at	At3g50060	MYB77	3.03
264119_at	At1g79180	Myb transcription factor	2.64
247535_at	At5g61620	Transcriptional activator-like	2.14
263735_s_at	At1g60040	MADS box protein	3.25
256050_at	At1g07000	Putative Leu zipper protein	2.14
261860_at	At1g50600	Scarecrow-like transcription factor 5 (SCL5)	2.30
		Transport Facilitation	
256833_at	At3g22910	Calcium-transporting ATPase	4.29
251176_at	At3g63380	Calcium-transporting ATPase	2.64
257183_at	At3g13220	ABC transporter	4.29

(*Table continues on following page.*)

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

Probe Set No.	AGI Locus	Gene Description	Fold Increase
254663_at	At4g18290	Potassium channel protein KAT2	2.46
253181_at	At4g35180	Similar to amino acid permease 1	3.25
245740_at	At1g44100	Amino acid permease 5 (AAP5)	2.00
250415_at	At5g11210	Ligand-gated channel subunit (GLR2.5)	3.03
254120_at	At4g24570	Mitochondrial uncoupling protein	2.00
245892_at	At5g09370	Putative lipid transfer protein	2.64
248392_at	At5g52050	MATE efflux protein-related	2.46
		Cellular Transport and Transport Mechanisms	
247493_at	At5g61900	Copine-like protein	4.29
251336_at	At3g61190	BON1-associated protein 1 (BAP1)	5.66
258786_at	At3g11820	Syntaxin (SYP121)	2.00
252053_at	At3g52400	Syntaxin (SYP122)	2.64
246453_at	At5g16830	Syntaxin (SYP21)	2.46
		Other Functions	
257206_at	At3g16530	Lectin-related	5.28
248686_at	At5g48540	33-kD secretory protein-related	3.48
250323_at	At5g12880	Putative Hyp-rich glycoprotein	2.83
254204_at	At4g24160	Putative protein CGI-58 protein-Homo sapiens	3.03
266884_at	At2g44790	Phytocyanin	3.25
266769_s_at	At2g03080	Putative reverse transcriptase	2.14
265618_at	At2g25460	SYNC 1 protein	2.30
247208_at	At5g64870	Nodulin-like	4.00
246927_s_at	At5g25260	Nodulin-like	2.46
252679_at	At3g44260	CCR4-associated factor 1-like protein	3.25
249928_at	At5g22250	CCR4-associated factor-like protein	2.83
255064_at	At4g08950	Phi-1 phosphate-induced protein-related	3.48
247280_at	At5g64260	Putative phi-1 protein	2.46
245757_at	At1g35140	Phosphate-induced 1 (PHI-1)	2.83
251884_at	At3g54150	Embryonic abundant protein-like	3.03
264758_at	At1g61340	Similar to late embryogenesis abundant protein	2.14
265075_at	At1g55450	Similar to embryo-abundant protein	3.03
254318_at	At4g22530	Embryo-abundant protein	2.14
252131_at	At3g50930	AAA-type ATPase	2.64
245765_at	At1g33600	Leu-rich repeat protein	3.03
252045_at	At3g52450	Similar to immediate-early fungal elicitor protein CMPG1	2.46
260706_at	At1g32350	Putative oxidase	2.30
259875_s_at	At1g76690	12-oxophytodienoate reductase (OPR2)	2.46
265938_at	At2g19620	Putative SF21 protein	2.64
254784_at	At4g12720	Growth factor-like protein	2.64

treatment (Simmons et al., 1992; Cheong et al., 2002). Callose (β-1, 3-glucan) accumulation was evident within 6 h of (β-D-Glc)$_3$ treatment (Fig. 2), and the induction of β-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 (β-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 (β-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 $\pm$ 0.48 (average of 3 trials $\pm$ SD) for the (β-D-Glc)$_3$ treatment and 1.36 $\pm$ 0.69 for the (β-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 (β-D-Glc)$_3$-induced callose deposition. To resolve this point, it would be interesting to test if (β-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+} (Schlüpmann et al., 1993; Li et al., 1997; Verma and Hong, 2001), so it is possible that the observed (β-D-Glc)$_3$-induction of callose

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)*

Probe Set No.	AGI Locus	Gene Description	Fold Increase
		Cell Rescue, Defense, Cell Death, and Aging	
252591_at	At3g45600	Similar to senescence-associated protein 5	2.00
267293_at	At2g23810	Similar to senescence-associated protein 5	2.46
262482_at	At1g17020	Senescence-related gene 1 (SRG1)	2.00
262930_at	At1g65690	Similar to hin1	5.28
263948_at	At2g35980	YLS9, hin1 homolog	2.46
250676_at	At5g06320	NDR1/HIN1-like protein 3 (NHL3)	2.14
247403_at	At5g62740	Hypersensitive-induced response protein	2.00
250483_at	At5g10300	Alpha-hydroxynitrile lyase-like protein	2.46
262455_at	At1g11310	MLO2	2.00
259297_at	At3g05360	Similar to Cf-2 LRR disease resistance protein	2.46
252102_at	At3g50970	Dehydrin Xero2	2.46
261749_at	At1g76180	Similar to dehydrin (ERD14)	2.00
258735_at	At3g05880	RC12A	2.83
258751_at	At3g05890	RC12B	2.30
251927_at	At3g53990	ER6 protein	2.00
264708_at	At1g09740	Putative ER6 protein	2.14
260986_at	At1g53580	Putative glyoxalase II	2.30
260408_at	At1g69880	Putative thioredoxin	2.14
246384_at	At1g77370	Putative glutaredoxin	2.14
263426_at	At2g31570	Glutathione peroxidase	2.00
257227_at	At3g27820	Putative monodehydroascorbate reductase	2.00
260943_at	At1g45145	Putative thioredoxin	2.14
266267_at	At2g29460	Putative glutathione *S*-transferase (GST22)	3.03
266290_at	At2g29490	Putative glutathione *S*-transferase (GST19)	2.46
266236_at	At2g02380	Putative glutathione transferase	2.46
260803_at	At1g78340	Putative glutathione transferase	3.25
260746_at	At1g78380	Putative glutathione transferase	2.00
258957_at	At3g01420	Alpha-dioxygenase (ALPHA-DOX1)	5.66
260060_at	At1g73680	Feebly-related protein	2.30
		Biogenesis of Plasma Membrane	
247851_at	At5g58070	Outer membrane lipoprotein-like	2.14
		Cell Wall	
254189_at	At4g24000	Cellulose synthase related (CSLG2)	2.46
265501_at	At2g15490	Glucosyltransferase-related	2.64
263221_at	At1g30620	UDP-Gal 4-epimerase-like protein (MUR4)	2.46
248100_at	At5g55180	Beta-1,3-glucanase-like	2.64
253559_at	At4g31140	Beta-1,3-glucanase-like	2.00
258805_at	At3g04010	Similar to beta-1,3-glucanase	22.63
245393_at	At4g16260	Similar to beta-1,3-glucanase	2.30
264685_at	At1g65610	Putative endo-1,4-beta-glucanase	2.00
259173_at	At3g03640	Putative beta-glucosidase	4.92
260130_s_at	At1g66280	Putative beta-glucosidase	3.48
253841_at	At4g27830	Putative beta-glucosidase	2.30
251427_at	At3g60130	Beta-glucosidase-like protein	3.25
251428_at	At3g60140	Beta-glucosidase-like protein	3.03
250142_at	At5g14650	Polygalacturonase-like protein	2.00
260492_at	At2g41850	Putative polygalacturonase	2.46
		Cytoskeleton	
265510_at	At2g05630	Putative microtubule-associated protein	2.14
265354_at	At2g16700	Actin depolymerizing factor 5 (ADF5)	2.00
246197_at	At4g37010	Similar to Caltractin	11.31
		Plant Development	
255794_at	At2g33480	Putative NAM	5.28
249467_at	At5g39610	NAM/CUC2-like protein	3.73
264148_at	At1g02220	NAM-like protein	3.03
261564_at	At1g01720	NAC domain protein (ATAF1)	2.46
260203_at	At1g52890	NAM-like protein	2.14
255585_at	At4g01550	NAM protein-related	2.00

(*Table continues on following page.*)

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

Probe Set No.	AGI Locus	Gene Description	Fold Increase
260156_at	At1g52880	NAM-like protein	2.00
250648_at	At5g06760	LEA-like	19.70
		Cellular Communication/Signal Transduction	
262099_s_at	At1g59500	Auxin-regulated protein GH3 related	2.00
248713_at	At5g48180	Similar to jasmonate inducible protein	3.73
250279_at	At5g13200	Similar to ABA-responsive protein	2.00
251665_at	At3g57040	Response reactor 4 (ARR4)	2.00
249741_at	At5g24470	Pseudo-response regulator 5 (APRR5)	2.46
266196_at	At2g39110	Putative protein kinase	2.64
247532_at	At5g61560	Putative protein kinase	2.00
259724_at	At1g60940	Similar to Ser/Thr proteinkinase ASK1	2.14
249771_at	At5g24080	Receptor-like protein kinase	3.25
251479_at	At3g59700	lecRK1	2.30
247957_at	At5g57050	ABA insensitive 2 (ABI2)	2.64
253994_at	At4g26080	ABA insensitive 1 (ABI1)	2.00
248132_at	At5g54840	SGP1 monomeric G-protein	2.30
259879_at	At1g76650	Calcium-binding EF-hand protein	2.14
260039_at	At1g68795	CLAVATA3/ESR-related 12 (CLE 12)	2.83
		Organization of Chromosome Structure	
262973_at	At1g75600	Similar to histone H3.2	2.30
262979_s_at	At1g75610	Similar to histone H3.2	2.83
		Ionic Homeostasis	
261410_at	At1g07610	Metallothionein-like protein	2.46
		Metabolism	
255521_at	At4g02280	Putative Suc synthetase	2.46
263157_at	At1g54100	Aldehyde dehydrogenase homolog	2.00
267168_at	At2g37770	Putative alcohol dehydrogenase	2.14
264953_at	At1g77120	Alcohol dehydrogenase (ADH1)	3.48
254197_at	At4g24040	Trehalase (ATRE1)	2.64
248381_at	At5g51830	Putative fructokinase	2.14
259442_at	At1g02310	(1–4)-beta-mannan endohydrolase	2.30
257866_at	At3g17770	Putative dihydroxyacetone kinase	2.30
249372_at	At5g40760	Glucose-6-phosphate dehydrogenase	2.00
258524_at	At3g06810	Putative acetyl-CoA dehydrogenase	2.30
267496_at	At2g30550	Lipase	2.00
256321_at	At1g55020	Lipoxygenase (LOX1)	2.83
260869_at	At1g43800	Putative stearoyl acyl carrier protein desaturase	2.64
261667_at	At1g18460	Similar to triacylglycerol lipase	2.46
260393_at	At1g73920	Similar to lipase	2.14
245249_at	At4g16760	Acyl-CoA oxidase 1 (ACX1)	2.00
266977_at	At2g39420	Putative phospholipase	3.03
266938_at	At2g18950	Tocopherol polyprenyltransferase (TPT1)	2.14
246502_at	At5g16240	Putative stearoyl-acyl carrier protein	2.14
256765_at	At3g22200	Gamma-aminobutyrate transaminase subunit (GABA-T)	2.46
250385_at	At5g11520	Asp aminotransferase (ASP3)	2.30
247729_at	At5g59530	1-aminocyclopropane-1-carboxylate oxidase-like	3.48
254630_at	At4g18360	Glycolate oxidase-like protein	2.00
265475_at	At2g15620	Ferredoxin-nitrite reductase (NIR1)	2.00
251973_at	At3g53180	Nodulin/Glu-ammonia ligase	2.00
253373_at	At4g33150	Lys-ketoglutarate reductase/saccharopine (LKR)	2.00
261957_at	At1g64660	Putative Met/cystathionine gamma lyase	2.46
251563_at	At3g57880	Anthranilate phosphoribosyltransferase-like protein	2.64
267207_at	At2g30840	Putative 2-oxoglutarate-dependent dioxygenase	2.14
265615_at	At2g25450	Putative dioxygenase	3.48
262616_at	At1g06620	Putative dioxygenase	2.30
		Secondary Metabolism	
248209_at	At5g53990	Flavonol 3-*O*-glucosyltransferase-like protein	3.03
247956_at	At5g56970	Cytokinin oxidase 3 (CKX3)	3.48
264042_at	At2g03760	Steroid sulfotransferase (RAR047)	2.46

(*Table continues on following page.*)

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

Probe Set No.	AGI Locus	Gene Description	Fold Increase
250662_at	At5g07010	Steroid sulfotransferase	2.30
246488_at	At5g16010	Steroid 5alpha-reductase-like protein	3.48
267147_at	At2g38240	Similar to flavonol synthase	2.46
259911_at	At1g72680	Putative cinnamyl-alcohol dehydrogenase (CAD)	2.14
261020_at	At1g26390	Similar to reticuline oxidase	2.83
261005_at	At1g26420	Similar to reticuline oxidase	2.30
		Cytochrome P450	
257623_at	At3g26210	CYP71B23	3.03
253046_at	At4g37370	CYP81D8	3.03
266155_at	At1g64950	CYP89A5	2.83
267559_at	At2g45570	CYP76C2	2.46
257035_at	At3g19270	CYP707A4	2.46
250752_at	At5g05690	CYP90A1 (DWF3)	2.30
252184_at	At3g50660	Steroid 22-alpha-hydroxylase (CYP90B1) (DWF4)	4.29
258114_at	At3g14660	CYP72A13	2.14
258094_at	At3g14690	CYP72A15	2.14
246984_at	At5g67310	CYP81G1	2.14
258064_at	At3g14680	CYP72A14	2.00
257634_s_at	At3g26170	CYP71B19	2.00
253096_at	At4g37330	CYP81D4	2.46
253052_at	At4g37310	CYP81H1	2.46
		Protein Destination	
262626_at	At1g06430	Putative FtsH protease	3.48
261240_at	At1g32940	Subtilisin-like Ser protease	2.46
245803_at	At1g47128	Cys proteinase RD21A	2.14
258005_at	At3g19390	Similar to Cys proteinase RD21A	3.03
260317_at	At1g63800	E2, ubiquitin-conjugating enzyme 5 (UBC5)	2.14
251104_at	At5g01720	Similar to F-box protein AtFBL3	2.14
253271_s_at	At4g34210	Similar to SKP1 homolog (ASK11)	2.00
266168_at	At2g38870	Putative protease inhibitor	2.46
250811_at	At5g05110	Cys proteinase inhibitor-like protein	2.30
245096_at	At2g40880	Cys proteinase inhibitor B (cystatin B)-related	4.29
		Transcription	
245252_at	At4g17500	AtERF1	2.14
252214_at	At3g50260	EREBP-3 homolog	2.00
260432_at	At1g68150	WRKY9	2.64
245976_at	At5g13080	WRKY75	2.64
253105_at	At4g35840	Zinc finger protein	2.46
257022_at	At3g19580	Cys-2/His-2-type zinc finger protein (AZF2)	2.00
246012_at	At5g10650	Pspzf zinc finger protein-like	3.25
260887_at	At1g29160	Dof zinc finger protein	2.30
248606_at	At5g49450	bZIP transcription factor	4.29
266555_at	At2g46270	G-box binding bZIP transcription factor (GBF3)	2.14
253245_at	At4g34590	G-box binding bZIP transcription factor (GBF6)	2.30
247199_at	At5g65210	bZIP transcription factor (TGA1)	2.14
246962_s_at	At5g24800	bZIP transcription factor (BZO2H2)	2.00
263956_at	At2g35940	Homeodomain-containing transcriptional factor (BLH1)	2.00
266346_at	At2g01430	Homeodomain-Leu zipper protein (ATHB-17)	2.00
251272_at	At3g61890	Homeobox-Leu zipper protein (ATHB-12)	2.64
252427_at	At3g47640	bHLH protein	2.00
265034_at	At1g61660	bHLH protein	2.83
262902_x_at	At1g59930	Putative MADS-box protein	2.14
261100_at	At1g63020	RNA polymerase IIA large subunit-related	2.00
		Transport Facilitation	
253630_at	At4g30490	Transport ATPase	2.14
251405_at	At3g60330	Plasma membrane H+-ATPase	2.46
247120_at	At5g65990	Amino acid transporter protein-like	2.30
264654_s_at	At1g08900	ERD6-related sugar transporter	2.14
245540_at	At4g15230	ABC transporter like protein	2.00

(*Table continues on following page.*)

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

Probe Set No.	AGI Locus	Gene Description	Fold Increase
251942_at	At3g53480	PDR5-like ABC transporter	4.00
263904_at	At2g36380	Putative ABC transporter	2.83
251962_at	At3g53420	Plasma membrane intrinsic protein 2a (PIP2A)	17.15
256833_at	At3g22910	Calcium-transporting ATPase	3.73
263699_at	At1g31120	Putative potassium transporter	6.96
264992_at	At1g67300	Putative hexose transporter	2.46
259133_at	At3g05400	Putative sugar transporter	2.14
250252_at	At5g13750	Transporter-like protein	2.46
250248_at	At5g13740	Transporter-like protein	2.83
245855_at	At5g13550	Sulfate transporter	2.30
245296_at	At4g16370	Oligopeptide transporter (OPT3)	2.46
		Cellular Transport and Transport Mechanisms	
254500_at	At4g20110	Vacuolar sorting receptor (AtELP3)	2.30
267547_at	At2g32670	VAMP725	2.00
255308_at	At4g04910	NSF	2.46
251975_at	At3g53230	CDC48-like protein	2.30
252027_at	At3g52850	AtELP1 homolog	2.00
251329_at	At3g61450	Syntaxin (SYP73)	4.29
		Other Functions	
266170_at	At2g39050	Similar to stress responsive lectin	2.64
249465_at	At5g39720	Avirulence induced gene AIG2-like protein	2.46
263161_at	At1g54020	Putative myrosinase-associated protein	2.00
254300_at	At4g22780	Uridylyltransferase-related	3.25
266203_at	At2g02230	SKP1 interacting partner 3-related	2.46
254559_at	At4g19200	Putative Gly/Pro-rich protein GPRP	3.25
253660_at	At4g30140	Putative protein Pro-rich protein APG	2.64
250918_at	At5g03610	Putative protein Pro-rich protein APG	2.14
263096_at	At2g16060	Class 1 nonsymbiotic hemoglobin (AHB1)	2.46
266884_at	At2g44790	Uclacyanin II (UCC II)	4.29
264751_at	At1g23020	Putative ferric-chelate reductase	2.83
250217_at	At5g14120	Nodulin-like protein	2.64
247488_at	At5g61820	Putative protein MtN19	2.64
264506_at	At1g09560	Germin-like protein	2.46
264365_s_at	At1g03220	Strong similarity to extracellular dermal glycoprotein (EDGP)	2.46
264219_at	At1g60420	Similar to tryparedoxin	2.46
261806_at	At1g30510	Putative ferrodoxin NADP oxidoreductase	2.46
257830_at	At3g26690	MutT-like protein	2.46
260042_at	At1g68820	C-term similar to C-term of apoptosis inhibitor	2.30
251743_at	At3g55890	Yippee-like protein	2.30
265482_at	At2g15780	Similar to blue copper protein	2.14
258880_at	At3g06420	Similar to symbiosis related proteins	2.14
255283_at	At4g04620	Putative symbiosis-related protein	2.00
253874_at	At4g27450	Similar to stem-specific protein	2.14
247307_at	At5g63860	UVB-resistance protein (UVR8)	2.14
264505_at	At1g09380	Putative nodulin protein	2.00
263211_at	At1g10460	Germin-like oxalate oxidase	2.00
252338_at	At3g48890	Putative progesterone-binding protein homolog (ATMP2)	2.00

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 (At*AGP18*, At*AGP21*, At*AGP22*; all with predicted GPI anchors) were up-regulated at least 2-fold at 1 h of $(\beta$-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). No AGP gene was

Table IV. *Comparison of Arabidopsis gene expression up-regulated by 50 μM (β-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.

AGI Locus	Gene Description	Fold Change			
		(β-D-Glc)$_3$-Induced Genes/1 h	(β-D-Glc)$_3$-Induced Genes/10 h	Wound-Induced Genes/30 min	Wound-Induced Genes/6 h
At4g33920	Putative protein phosphatase	2.00		6.15	
At4g23190	RLK3	2.14		5.61	
At4g23180	RLK4	2.14		2.41	
At4g34390	Extra-large G-protein-like	2.00		3.66	
At4g39890	Ras family GTP-binding protein	2.00		3.55	
At5g54840	SGP1 monomeric G-protein		2.3		3.18
At1g22810	TINY-like transcription factor	2.46		4.78	
At4g17500	AtERF1		2.1	5.71	
At5g47230	AtERF5	2.00		6.42	
At4g17490	AtERF6	4.92		5.77	
At1g28370	Similar to AtERF11	2.30		20.61	
At1g68840	RAV2	2.64		3.74	
At4g25470	DREB1C/ CBF2	2.30		2.14	
At2g38470	WRKY33	2.64		15.12	
At1g80840	WRKY40	3.25		25.52	
At4g23810	WRKY53	2.30		13.24	
At1g27730	ZAT10/ STZ	3.25		10.21	
At2g37430	ZAT11	128.00		18.91	
At5g59820	ZAT12	4.92		11.64	2.17
At3g28210	Zinc finger protein (PMZ)	2.14		4.05	3.31
At3g54810	GATA transcription factor 3	2.46		3.01	
At3g23250	MYB15	3.03		17.88	
At1g18570	MYB51	3.03		8.42	
At4g37260	MYB73	2.14		3.36	
At3g50060	MYB77	3.03		7.91	
At3g61890	Homeobox-Leu zipper protein ATHB-12		2.6		2.28
At4g25810	At-XTH23 (XTR6)	3.03		10.50	
At5g57560	At-XTH22 (TCH4)	2.14		4.08	2.18
At1g65310	At-XTH17 (XTR1)	5.66		4.06	
At3g50760	Glycosyltransferase	2.14		2.46	
At4g02330	Similar to pectinesterase	3.73		3.37	3.41
At2g27500	Putative beta-1,3-glucanase	2.00		3.61	
At4g24000	Cellulose synthase-related (CSLG2)		2.5		4.88
At1g30620	UDP-Gal 4-epimerase-like (MUR4)		2.5	2.18	2.15
At2g43620	Putative endochitinase	2.30			16.35
At2g40000	Nematode-resistance protein related	4.59		12.08	
At3g44630	Disease resistance protein RPP1-WsB-like	2.00		2.13	
At2g34930	LRR disease resistance protein	3.48			4.88
At3g50970	Dehydrin Xero2	2.46	2.5		15.36
At2g23810	Similar to senescence-associated protein 5	2.30	2.5	4.31	
At2g38870	Protease inhibitor related	5.66	2.5		4.59
At5g39580	Peroxidase ATP24a	4.59		15.41	
At5g47910	Respiratory burst oxidase protein D, RBOHD	2.64		4.81	
At2g29460	Putative glutathione S-transferase (GST22)		3.0		3.28
At2g45570	CYP76C2		2.5		4.22
At4g20860	Similar to reticulin oxidase	2.30		2.31	3.27

induced more than 2-fold at 10 h of (β-D-Glc)$_3$ treatment, but *AtAGP18*, *AtAGP22*, *AtFLA1*, and At*FLA9* 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 (β-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 (β-D-Glc)$_3$ treatment. Several apparent glycosyltransferases of unknown substrate specificity were induced at 1 h (Table II). Two cellullose synthase-related genes were moderately up-regulated, At4g07960 at 1 h (Table II) and 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 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).

Probe Set No.	AGI Locus	1 h (β-D-Glc)$_3$			10 h (β-D-Glc)$_3$		
		Signal	Fold Change	Expression Change	Signal	Fold Change	Expression Change
263183_at	At1g05570	599.5	0.93	NC	330.7	1.07	NC
265729_at	At2g31960	773.8	0.81	D	771.5	1.62	I
250272_at	At5g13000	1,387	0.87	NC	3,066.5	1.07	NC
264112_at	At2g13680	233.7	1.00	NC	146	1.52	NC
262628_at	At1g06490	208.7	1.00	NC	165.9	1.15	NC
258122_at	At3g14570	138.3	1.00	NC	151	1.23	NC
258826_at	At3g07160	1,810.5	1.00	NC	3,496	1.32	I
255378_at	At4g03550	1,231.3	1.07	NC	2,856	1.23	I
255281_at	At4g04970	421.7	1.07	NC	638.3	1.15	NC
251499_at	At3g59100	221.6	1.15	NC	415.3	1.41	I
249635_at	At5g36870	149.6	1.15	NC	162.6	1.00	NC
263891_at	At2g36850	1,602.2	1.00	NC	2,280	1.62	I

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*, *DREB1C/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 Cys_2/His_2-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 (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 $^{L}/_{F}DLN^{L}/_{F}(X)P$ in their C-terminal region (Ohta 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

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

The type of AGP is indicated by a letter (C, classical AGP; P, AG-peptide; K, Lys-rich AGP; F, fasciclin-like AGP) following the gene name, and the font of this letter indicates whether the AGP is predicted to have a GPI-anchor (italicized font, without GPI-anchor; regular font, with GPI anchor; Schultz et al., 2002; Johnson et al., 2003). Detection calls in columns labeled Dc were determined using default values in the Microarray Suite (MAS) 5.0 software (Affymetrix). See Table V caption for details on other columns. P, present; A, absent; M, marginal; NC, no change; I, increase; D, decrease. Note that *AtAGP19*, *AtFLA4*, *AtFLA5*, and *AtFLA17* are not annotated in the ATH1 array.

Gene Name	Probe Set No.	AGI Locus	1 h (β-D-Glc)$_3$				10 h (β-D-Glc)$_3$			
			Signal	Dc	Fold Change	Expression Change	Signal	Dc	Fold Change	Expression Change
AGP1/ C[a]	247279_at	At5g64310	15,126.9	P	1.15	NC	16,821.1	P	1.00	NC
AGP2/ C[a]	264005_at	At2g22470	6,549.2	P	1.32	I	6145	P	1.87	I
AGP3/ C	252833_at	At4g40090	57.7	A	1.41	NC	49.8	A	1.52	NC
AGP4/ C	250427_at	At5g10430	50.7	A	1.74	NC	4.8	A	0.31	NC
AGP5/ C	259550_at	At1g35230	41.3	A	4.29	I	13.5	A	0.23	NC
AGP6/ C	250174_at	At5g14380	121.4	P	1.07	NC	97.8	P	0.87	NC
AGP7/ C	247189_at	At5g65390	498	P	1.15	NC	488.3	P	1.52	I
AGP9/ C[a]	266588_at	At2g14890	6,418	P	1.15	NC	7,667.4	P	0.87	NC
AGP10/ C[a]	255080_at	At4g09030	8,068.4	P	1.32	I	12,507	P	1.07	NC
AGP11/ C	259180_at	At3g01700	78.8	A	0.87	NC	52.2	A	0.66	NC
AGP25/ C	250002_at	At5g18690	95.3	P	0.87	NC	49.8	A	0.47	D
AGP26/ C	266460_at	At2g47930	11.3	A	0.20	NC	15.2	A	0.93	NC
AGP27/ C	258914_at	At3g06360	139.9	P	0.87	NC	327.9	P	0.71	NC
AGP12/ P[a]	256964_at	At3g13520	7,993.9	P	1.32	I	13,602.4	P	1.15	NC
AGP13/ P	253957_at	At4g26320	81.5	A	1.15	NC	34.7	A	0.29	D
AGP14/ P	247965_at	At5g56540	329.1	P	0.93	NC	97.2	P	0.71	NC
AGP15/ P[a]	250358_at	At5g11740	13,631.3	P	1.15	NC	18,429.9	P	0.93	NC
AGP16/ *P*[a]	266552_at	At2g46330	10,002.8	P	1.41	I	4,532.2	P	0.93	NC
AGP20/ *P*	251281_at	At3g61640	2241.7	P	1.32	I	861.5	P	1.00	NC
AGP21/ P[a]	259664_at	At1g55330	7,774.6	P	2.00	I	4,782.8	P	0.93	NC
AGP22/ P	248252_at	At5g53250	1,257.9	P	2.83	I	62.8	A	0.22	D
AGP23/ P	251590_at	At3g57690	298.1	P	1.00	NC	323.3	P	1.00	NC
AGP24/ P	249375_at	At5g40730	1,281.3	P	1.23	I	3,533.2	P	1.62	I
AGP17/ K	267260_at	At2g23130	125.6	M	1.15	NC	46.4	A	0.71	NC
AGP18/ K[a]	253050_at	At4g37450	5,237.4	P	2.30	I	1,289.9	P	0.41	D
FLA1/ F	248074_at	At5g55730	784.6	P	0.81	NC	219	P	0.23	D
FLA2/ F	254785_at	At4g12730	1,148.3	P	1.32	I	2,330.1	P	0.66	D
FLA3/ F	257392_at	At2g24450	41.9	A	3.25	NC	14.8	A	1.00	NC
FLA6/ F	263376_at	At2g20520	14.9	A	1.23	NC	9.9	A	1.15	NC
FLA7/ F	263628_at	At2g04780	1,495.8	P	1.23	I	1,752.2	P	1.00	NC
FLA8/ F	251395_at	At2g45470	135.8	A	0.81	NC	264.9	P	0.71	D
FLA9/ F	265066_at	At1g03870	219.6	A	2.14	NC	130.3	P	0.44	D
FLA10/ F	251394_at	At3g60900	50.1	A	1.32	NC	28.3	A	0.62	NC
FLA11/ F	250933_at	At5g03170	87.6	A	0.93	NC	67.7	A	0.62	NC
FLA12/ F	247638_at	At5g60490	163.9	P	0.93	NC	233	P	1.41	I
FLA13/ F	249037_at	At5g44130	186.4	P	1.23	NC	139.4	P	0.81	NC
FLA14/ F	257691_at	At3g12660	105.8	A	1.23	NC	54.3	A	0.76	NC
FLA15/ *F*	256673_at	At3g52370	38.7	A	1.07	NC	76.1	M	2.00	NC
FLA16/ *F*	263942_at	At2g35860	625.2	P	1.41	I	953.5	P	0.62	D
FLA18/ *F*	259072_at	At3g11700	2,698.6	P	1.62	I	1,273.2	P	0.71	D
FLA19/ *F*	262606_at	At1g15190	226.2	A	1.07	NC	25.8	A	0.23	NC
FLA20/ *F*	249323_at	At5g40940	19.9	A	0.87	NC	4.4	A	0.11	NC
FLA21/ *F*	250652_at	At5g06920	68	A	0.87	NC	47.6	A	2.14	NC

[a]Indicates the more abundant AGPs in the cell culture.

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)$_3$.

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

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

Each fold change shown for a real-time PCR measurement is the average of three, or occasionally two, trials ±SD.

Gene Description	AGI Locus	Treatment	Fold Change			
			Microarray	Real-Time PCR[a]	Replication 1[b]	Replication 2[b]
ZAT11	At2g37430	1 h (β-D-Glc)$_3$	128	122.41 ± 8.20	11.35 ± 2.09	3.82 ± 0.31
		1 h (β-D-Man)$_3$	11.31	6.94 ± 2.66	1.37 ± 0.04	1.38 ± 0.22
AtMPK3	At3g45640	1 h (β-D-Glc)$_3$	4.59	4.33 ± 0.03	1.35 ± 0.13	1.60 ± 0.24
TINY-like	At1g33760	1 h (β-D-Glc)$_3$	9.19	4.61 ± 0.28	9.77 ± 0.97	4.29 ± 0.80
β-1,3-glucanase	At5g55180	10 h (β-D-Glc)$_3$	2.64	3.40 ± 0.19	1.35 ± 0.55	1.62 ± 0.37
Callose synthase	At3g07160	1 h (β-D-Glc)$_3$	1	1.22 ± 0.16	0.76 ± 0.08	1.01 ± 0.46
		1 h (β-D-Man)$_3$	1	0.79 ± 0.06	0.70 ± 0.05	0.77 ± 0.04
		10 h (β-D-Glc)$_3$	1.32	1.42 ± 0.10	1.10 ± 0.09	1.32 ± 0.32

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

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 AGPs. The (β-D-Glc)$_3$ treatment induced three lectin receptor protein kinase genes (At4g29050, At5g01540, At1g70130; Table II) at 1 h and another (At3g59700; Table III) at 10 h. The At3g59700 gene, encoding the LecRK-1 lectin receptor protein kinase, has been shown to be expressed during senescence and wounding (Riou et al., 2002). Thus, the expression of *LecRK-1* in the current experiments may reflect a (β-D-Glc)$_3$-induced wound-like response or cell death (Fig. 1). The cell wall-associated kinases (WAK) have been shown to play a role in cell expansion and in plant defense. WAK1 has been shown be covalently bound to pectins (Verica and He, 2002). Two WAK-like genes (At1g16130, At1g79680; Table II) were slightly induced by (β-D-Glc)$_3$ at 1 h. Other receptor-like protein kinase genes induced at 1 h of (β-D-Glc)$_3$ treatment included *RLK3* and *RLK4*, which were previously shown to be induced by pathogen invasion, salicylic acid, and wounding (Table IV; Czernic et al., 1999; Cheong et al., 2002).

The (β-D-Glc)$_3$ treatment increased the expression of 17 protein kinases at 1 h (Table II). By 10 h of treatment, only 3 protein kinases had higher expression levels (Table III), but many had lower expression levels (Supplemental Table III). The most highly induced, previously identified protein kinase gene was *AtMPK3*, which was induced 4.59-fold at 1 h of (β-D-Glc)$_3$ treatment. The AtMPK3 kinase has been well studied at the levels of both gene expression and enzyme activity, the regulation of the latter potentially involving both transcriptional and posttranscriptional mechanisms (Mizoguchi et al., 1996; Ichimura et al., 2000; Zhang and Klessig, 2001). Earlier reports (Kovtun et al., 2000; Asai et al., 2002) have shown that AtMPK3 kinase activity is induced by pathogen elicitors and H_2O_2, whereas *AtMPK3* gene expression is induced upon incompatible interaction with the necrotrophic fungus *Alternaria brassicicola* (Schenk et al., 2003). Both gene expression and enzyme activity of WIPK, a tobacco ortholog of AtMPK3, are induced by wounding (Seo et al., 1995, 1999). Changes in expression of many receptor-like protein kinases and other protein kinases within the first 1 or 10 h present possible candidates for the transduction of (β-D-Glc)$_3$ binding of cell surface AGPs into downstream cellular responses.

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 characteristically early and transient, since very few of these genes were induced above 2-fold at 10 h (Table III). Plant *R* genes are involved 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, phenylanaline 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

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 inactivates 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

no loss of viability when continuously exposed to (β-D-Glc)$_3$ for a 7-d period. This observation very strongly argues that (β-D-Glc)$_3$ is not inherently toxic or damaging to plant cells. Much more likely than membrane tearing is the hypothesis that aggregation of AGPs by (β-D-Glc)$_3$ initiates a signal transduction event. This signal transduction hypothesis is also consistent with the demonstration that death of Arabidopsis cells treated with (β-D-Glc)$_3$ occurs via programmed cell death (Gao and Showalter, 1999).

If aggregation of AGPs by (β-D-Glc)$_3$ is a signal transduction event, then the AGPs might be components in the transduction pathway. Most genes annotated as encoding an AGP core polypeptide predict the presence of a GPI-anchor, and this anchor may be integral to hypothesized AGP functions in cell-cell signaling (Schultz 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 (Peskan 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.

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)$_3$ or 50 μM (β-D-Man)$_3$.

Histochemistry

Callose in Arabidopsis cells was visualized by fluorescence staining with Aniline Blue, or more accurately with Sirofluor, a callose-staining fluorochrome 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 fluorogenic 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 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-(dT)$_{24}$ 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 Diagnosis, 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-phycoerythrin on an Affymetrix Fluidics Station 400 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 values).

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: At*MAPK3*, 5′-gacagagttgcttggcacacc, 5′-gctaagggctgacgtggga; *ZAT11*, 5′-gagattttcttcgtttcaagccc, 5′-ttcacatctttctgctcaacgg; *TINY-like*, 5′-cggaaagctctagttcggagc, 5′-ttgaccagactcgagagctgg; *β-1,3-glucanase*, 5′-ttcaggaaggtctcgactacgc, 5′-accaccaaaattacacgtgcc; *callose synthase (At3g07160)*, 5′-tctttctattgcgtgtgcttgg, 5′-ggaaggtcgacacaaaaggg; *callose synthase (At4g03550)*, 5′-attcaggtcgggaagggac, 5′-cggtacacatctcggctgag; and *actin 2/7*, 5′-ctcatgaagattctcactgag, 5′-acaacagatagttcaattccca. 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.

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