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Global Transcription Profiling Reveals Multiple Sugar Signal Transduction Mechanisms in Arabidopsis [W]

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Complex and interconnected signaling networks allow organisms to control cell division, growth, differentiation, or programmed cell death in response to metabolic and environmental cues. In plants, it is known that sugar and nitrogen are critical nutrient signals; however, our understanding of the molecular mechanisms underlying nutrient signal transduction is very limited. To begin unraveling complex sugar signaling networks in plants, DNA microarray analysis was used to determine the effects of glucose and inorganic nitrogen source on gene expression on a global scale in *Arabidopsis thaliana*. In whole seedling tissue, glucose is a more potent signal in regulating transcription than inorganic nitrogen. In fact, other than genes associated with nitrate assimilation, glucose had a greater effect in regulating nitrogen metabolic genes than nitrogen itself. Glucose also regulated a broader range of genes, including genes associated with carbohydrate metabolism, signal transduction, and metabolite transport. In addition, a large number of stress responsive genes were also induced by glucose, indicating a role of sugar in environmental responses. Cluster analysis revealed significant interaction between glucose and nitrogen in regulating gene expression because glucose can modulate the effects of nitrogen and vise versa. Intriguingly, cycloheximide treatment appeared to disrupt glucose induction more than glucose repression, suggesting that de novo protein synthesis is an intermediary event required before most glucose induction can occur. Cross talk between sugar and ethylene signaling may take place on the transcriptional level because several ethylene biosynthetic and signal transduction genes are repressed by glucose, and the repression is largely unaffected by cycloheximide. Collectively, our global expression data strongly support the idea that glucose and inorganic nitrogen act as both metabolites and signaling molecules.

INTRODUCTION

Plants can sense levels of nutrients and accordingly adjust growth and development. The perception mechanisms are complex regulatory circuits that control gene expression to accommodate constant changes of nutrient-dependent cellular activities. Reduced carbon is essential both as a building block and as an energy source for all organisms. Uniquely, plants generate their own reduced carbon through photosynthesis (Yunus et al., 2000). Nitrogen is a necessary component of many biosynthesized molecules—plants typically acquire it in the form of inorganic nitrate from the soil (Marschner, 1995). To adapt to environmental and metabolic cues, complex regulatory networks have been used by different organisms to sense nutrient signals and regulate gene expression (DeRisi et al., 1997; Wang et al., 2000; Lin et al., 2002; Shalev et al., 2002; Zinke et al., 2002; Boer et al., 2003; Buckhout and Thimm, 2003; Wang et al., 2003) In plants, elevated levels of cellular sugar upregulate genes involved in the synthesis of polysaccharides, storage proteins, pigments, as well as genes associated with defense responses and respiration. By contrast, sugar deprivation enhances the expression of genes involved in photosynthesis and resource remobilization, such as the degradation of starch, lipid, and protein (Koch, 1996; Yu, 1999; Ho et al., 2001). While it seems that a profound number of genes are regulated by sugars, the underlying molecular mechanisms of sugar signaling are poorly understood. So far, only a handful of *cis*-regulatory elements and *trans*-acting factors required for a sugar response have been identified (Yu, 1999; Lu et al., 2002; Rolland et al., 2002). Because multiple sugar signal transduction pathways exist in plants, additional *cis*-elements, *trans*-acting factors, and upstream receptors and signaling components are expected to be involved in regulatory networks that transmit sugar signals. Therefore, a high throughput approach is needed to systematically identify these signaling molecules and their mode of actions in sugar-regulated gene expression in plants.

Sugars such as glucose and sucrose can act as signals that trigger changes in gene expression in plants. Using a maize (*Zea mays*) protoplast transient expression assay, it was found that glucose-regulated photosynthetic gene expression requires both membrane-bound sugar transporter and hexokinase (HXK) (Jang and Sheen, 1994). However, hexose phosphorylation is not required for the induction of genes encoding extracellular invertase, sucrose synthase, or storage protein (Roitsch et al., 1995; Martin et al., 1997). Based on the expression

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patterns of 26 genes in various cellular functions, it has been revealed that three distinct glucose signaling pathways exist in plants: (1) an AtHXK-dependent, (2) a HXK enzymatic activity-dependent (irrespective of AtHXK or yeast YHXK2), and (3) a HXK-independent (Xiao et al., 2000) pathway. Similar results were obtained using rice (*Oryza sativa*) cell cultures where transcription rate and mRNA stability were shown to be affected by sugars (Ho et al., 2001), illustrating a diverse role of sugar in gene regulation. A recent microarray study measuring the effects of sucrose and light using the Affymetrix AG chip (having 8000 unique targets) revealed that genes associated with metabolism, protein synthesis/modification, and energy were overrepresented when compared with genes unaffected by the treatments (Thum et al., 2004).

Nitrogen sources, such as NO_3^-, have been shown to regulate gene expression associated with nitrogen uptake/incorporation and starch metabolism (Forde, 2002; Stitt et al., 2002); however, the presence of sugar also affects transcription of genes (Lam et al., 1998) and posttranslational modification of proteins (Cotelle et al., 2000) associated with nitrogen metabolism. For instance, the transcription of Asn synthetase and Glu dehydrogenase gene is downregulated by sugar (Melo-Oliveira et al., 1996; Lam et al., 1998). These results have implicated a model in which genes involved in carbon and nitrogen metabolism are cross-regulated by both carbon and nitrogen signals (Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001). An earlier DNA microarray analysis measuring global gene responses to nitrate treatment confirmed that genes associated with nitrate uptake, nitrite reduction (into NH_4^+), and ammonium assimilation were upregulated when *Arabidopsis thaliana* seedlings were treated with exogenous nitrate (Wang et al., 2000). Another more recent study using seedlings grown hydroponically until the exogenously applied ammonium became depleted revealed that glycolysis-related genes were upregulated in roots upon brief treatment with nitrate (Wang et al., 2003). It is yet to be determined whether sugar plays a role in nitrate-induced global gene expression change.

Microarray technology using synthesized oligomer probes permits the analysis of thousands of Arabidopsis genes in a single experiment with small amounts of RNA template (Epstein and Butow, 2000; Schaffer et al., 2000); newer microarrays like the Affymetrix ATH1 GeneChip can measure expression in virtually the whole genome (Zhu, 2003). In this study, we investigate the effects of exogenous glucose on global gene expression in Arabidopsis seedlings using the ATH1 GeneChip. Using control samples free of exogenous sugar or nitrogen, we were able to identify the individual contributions of sugar, nitrogen, or sugar plus nitrogen on global gene expression. Our results show that glucose is a surprisingly potent signal for transcriptional regulation, affecting a broad range of gene classes. We also find that transcriptional cascades are involved in sugar regulatory response and that glucose repression is a more direct process than glucose induction.

RESULTS

To determine the effects of exogenous sugar and/or nitrogen on gene expression, we analyze the expression of RNA from whole seedlings using the 22,500+ gene ATH1 Arabidopsis GeneChips as target probe sets. Because sugars can delay the onset of germination compared with the control (Price et al., 2003), sugar treatment may conceivably have two general impacts: alteration of gene expression that is sugar specific and changes in gene expression that are developmentally or temporally regulated. To minimize the impact of the developmental program, we grew the plants for 5 d in MS liquid medium with 58.4 mM sucrose to allow all the plant material to be at approximately the same developmental stage. We then washed all seedlings and maintained them in the dark for 24 h in sugar- and nitrogen-free MS to reduce the endogenous sugar and nitrogen. This was followed by the experimental treatment: a 3-h pulse in the dark with either added sugar, nitrogen source, both sugar and nitrogen source, sugar analog 3-*O*-methylglucose, or control additive (water). Total RNA was prepared after the pulse, and this was used to make biotinlylated probe for the GeneChip hybridization.

The timing and concentration of sugar or nitrogen pulse was largely based on prior and preliminary experiments. A pilot experiment was conducted to show that a 24-h deprivation period without carbon or nitrogen source was sufficient to see significant transcriptional changes. A longer period was not selected because we are interested in transient regulatory events—in Arabidopsis, a different set of responses have been shown to occur upon prolonged nitrogen starvation (Lejay et al., 1999). The nitrogen added, 40 mM nitrate and 20 mM NH_4^+, was identical to the nitrogen sources present in MS salts (GIBCO, Invitrogen, Grand Island, NY), a universal growth medium employed and cited in numerous plant studies. We chose to use glucose as the carbon source because glucose is a potent regulator for gene expression, growth, and development (Rolland et al., 2002). Glucose at 167 mM maximally affected the transcription of abscisic acid (ABA)-related genes *ABA2*, *ABI1*, and *ABI4* when compared either to the control or higher levels of glucose (Price et al., 2003). The glucose analog 3-*O*-methylglucose (3-OMG) served as a control because it can be transported into the cell like glucose, but because it cannot signal upon phosphorylation by HXK (Cortes et al., 2003), it distinguishes HXK-independent glucose signal transduction from HXK-dependent and glycolysis-dependent (via HXK activity) glucose signaling pathways (Xiao et al., 2000). All treatments were compared with a carbon- and nitrogen-free control containing mock additive (water). Four independent biological replicates were conducted for the treatments above, using pooled plant material for each sample but not pooling material between replicates.

To assess the quality of the data, scatter plots comparing one control replicate with another were completed to determine if the plots were linear (with slope = 1) and had a compact distribution. Graphs of all possible replicate pairs were generated for the controls; a typical normalized example is presented in Supplemental Figure 1A online. Graphs of experimental replicate versus correspondingly treated replicate were similar in appearance to the control graphs (data not shown). None of our data appeared to have nonlinear bias before normalization, so we used MicroArray Suite 5.0 to conduct scalar normalization of the data (Bolstad et al., 2003). Plots of the $\log_2$ average signal versus $\log_2$ signal difference comparing two control normalized replicates showed that the data were linearly distributed with an average

slope = 0 (see Supplemental Figure 1B online), confirming that the scalar normalization with MicroArray Suite was appropriate for our analyses. Randomized complete block design (RCBD) analysis (Shieh and Jan, 2004) was conducted on $\log_{10}$ normalized data at $P \leq 0.001$, resulting in an estimated false positive rate of 23 genes. The false discovery rate (FDR) for our data was also calculated as described by Storey and Tibshirani—the FDR ranged from approximately six false positives for glucose-treated and glucose and nitrogen–treated samples to 22 for plants treated with nitrogen alone at $P \leq 0.001$ (Storey and Tibshirani, 2003) (see Supplemental Figures 2A to 2D online). To further reduce the occurrence of false positives, a threefold cutoff filter was applied for most subsequent analyses, whereas twofold filtering was applied in some instances where more comprehensive lists of regulated genes were desirable.

Effect of Nitrogen on Transcriptional Patterns

A previous microarray study using exogenous nitrate (Wang et al., 2000) revealed a relatively short list of genes that had altered transcriptional patterns. This study compared genes that were transcriptionally regulated by low (250 μM) and high (10 mM) nitrate levels when supplemented with 0.5% sucrose as a carbon source. Out of ~5500 unique genes, 49 showed a twofold or greater change in mRNA levels. A more recent microarray analysis measuring nitrate response in Arabidopsis suggested a larger number of genes were regulated by nitrate (Wang et al., 2003). In the latter experiment, plants were grown in medium containing 0.5% sucrose, and plants were allowed to deplete their sole nitrogen source, 2.5 mM ammonium succinate, over a 10-d period before being treated with 250 μM KNO_3 for 20 min. Using a twofold cutoff and the Wilcoxon's signed rank scores of I (increase) or D (decrease), it was found that 251 genes were induced and 78 genes were repressed in root tissue, whereas in shoot tissue 76 genes were induced and two were repressed. The major differences between the two studies were that in the latter study, the roots were analyzed separately from the shoot tissue and the period of nitrogen starvation was longer. In our study, we used whole plants, in which shoot mass outweighed root mass by 22.8-fold, no carbon source was supplied, and a relatively short period (24 h) of nitrogen deprivation was used. To exclude targets with inconsistent results, we used an RCBD analysis cutoff of $P \leq 0.001$ with a twofold change to filter our data. When we tested the effects of higher concentrations of nitrogen (40 mM nitrate and 20 mM NH_4^+; standard for MS medium) using a sugar-free medium, only 106 and 129 genes showed greater than twofold induction or repression, respectively (Figure 1; see Supplemental Table 1 online). When an additional filter was applied to eliminate genes with expression near background levels, 24 upregulated and 37 downregulated genes were selected as nitrogen regulated. Some of the nitrogen-regulated genes were identified in the earlier microarray studies as being associated with nitrate/nitrite assimilation (Wang et al., 2000; Wang et al., 2003): among these were nitrate reductase 1 (NIA1), urophorphyrin III methylase, and ferredoxin nitrite reductase (Table 1). Markedly, two genes associated with ammonium assimilation in shoots, Asn synthetase (ASN2) and NADH-dependent Glu synthase (Temple et al., 1998; Wong et al., 2004), showed stronger upregulation in our study compared with the latter nitrate microarray study (Table 1) (Wang et al., 2003), presumably because we restored both nitrate and ammonium to our nitrogen-deprived plants.

We also examined the effects of nitrogen treatment when glucose was also supplied. Our results revealed that the induction of many of the previously reported nitrate-responsive genes actually required the presence of both nitrogen and sugar (Table 1), suggesting an interaction between sugar and nitrogen. The interaction is further supported by the results of cluster

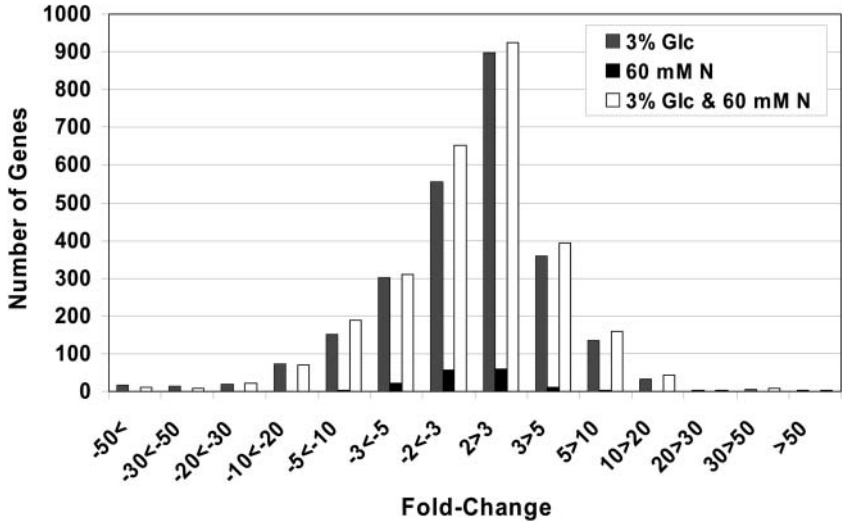

Figure 1. Glucose Has Profound Effects on Gene Expression Compared with Inorganic Nitrogen in 6-d-Old Arabidopsis Seedlings Predominantly Consisting of Shoot Tissue.

To remove inconsistent replicates, $\log_{10}$ normalized signal scores were subjected to RCBD analysis ($P \leq 0.001$) before twofold filtering.

Table 1. A Comparison of Nitrate-Regulated Gene Expression between Wang et al. (2003) and This Study

		Wang et al. (2003)	Fold-Change Ratios		
Probe Set ID	Gene Description	Nitrate/Control Ratio	Glc/Control	N/Control	Glc and N/Control
260623_at	Nitrate transporter (NRT2.1)	19.6[a]	NC	NC	NC
259681_at	Nitrate reductase (NIA1)	3.2	1.1	19.5	19.6
261979_at	Nitrate reductase (NIA2)	2.4	−3.0	1.8	1.3
265475_at	Nitrite reductase (NiR)	24.3	8.0	7.3	30.0
249325_at	Urophorphyrin III methylase	13.5	2.3	2.6	14.2
255230_at	Ferredoxin NADP reductase	4.2	5.3	1.5	21.9
261806_at	Ferredoxin NADP reductase	4.8	1.9	−1.1	8.8
265649_at	Putative ferredoxin	2.8	1.9	1.3	5.0
264859_at	Glucose-6-phosphate 1-dehydrogenase	36.3	4.0	1.1	62.0
245977_at	Glucose-6-phosphate 1-dehydrogenase	5.1	1.6	1.1	7.6
249266_at	6-Phosphogluconate dehydrogenase	5.2	3.5	−1.0	12.3
262323_at	6-Phosphogluconate dehydrogenase	2.6	1.4	−1.0	3.0
248267_at	Glu synthase (GOGAT NADH)	1.6	1.8	2.3	4.6
247218_at	Asn synthetase (ASN2)	2.0	2.6	9.7	30.6
262180_at	Phosphoglycerate mutase	32.3	8.2	1.5	35.2
264246_at	Trehalose-6-phosphate synthase	NC	−5.1	1.4	−3.4
263019_at	Trehalose-6-phosphate synthase	NC	−19.7	1.4	−10.9
257217_at	Phosphoenolpyruvate carboxylase (PPC)	2.1	1.6	−1.0	2.0
252407_at	Chloroplast malate dehydrogenase	2.1	2.0	−1.0	3.6

Shoot data rather than root data were used (Wang et al., 2003) for comparison because shoot tissue was overrepresented in our whole plant samples collected for analysis. NC, no change.

[a] Expression signal near background levels.

analysis: ~8% of glucose-responsive genes showed altered expression when nitrogen was also present (Figure 2, clusters 1, 7, and 8). Only a few of these genes, primarily those in clusters 1 and 7, were regulated by nitrogen alone. Glucose and nitrogen appear to have synergistic effects on the induction of some genes (Figure 2, cluster 8). For example, uroporphyrin III methyltransferase (*UPM1*) and glucose-6-phosphate dehydrogenase (264859_at) showed modest induction in the presence of either glucose or nitrogen, but the combination of both nutrients increased expression far greater than if the effect of each nutrient were merely additive (Table 1). For *UPM1*, this synergistic effect was verified by RNA gel blot analysis (Figure 3C). In other examples, the regulation of gene expression occurred via an antagonistic interaction between C and N signals (Figure 2, cluster 1). A stress-related gene previously identified as *SAG21* (At4g02380) was reported to be upregulated 4.5-fold by nitrogen when compared with a control containing sucrose (Wang et al., 2000); we observed that nitrogen without glucose minimally regulated *SAG21* (1.3-fold) but also found that the gene was downregulated by glucose 4.6-fold when compared with a C- and N-free control (Figure 3D). When sugar and nitrogen were both available, nitrogen derepressed the glucose effect and brought transcription of *SAG21* near to the C- and N-free control levels (down 1.4-fold). Of the 61 nitrogen-regulated genes showing a more than twofold expression change, more genes were found to be downregulated by nitrogen than upregulated—this is not observed in previous microarray studies. This difference is likely attributable to the presence of sugar in the earlier experiments and the use of a C- and N-free control under our conditions.

Effect of Glucose on Transcriptional Patterns

In contrast with nitrogen, glucose was more potent in regulating transcription under the conditions we used (Figure 1). Of genes regulated by carbon and/or nitrogen, cluster analysis revealed that glucose altered transcription for a large portion of genes, whereas nitrogen treatment had little to no effect (Figure 2, clusters 0, 2, 3, 5, and 6). However, nitrogen could modulate the glucose effect for a smaller subset of genes (Figure 2, clusters 1, 7, and 8). Using an RCBD analysis cutoff of $P \leq 0.001$ and a threefold change to filter our data, 534 and 444 genes were found to be downregulated and upregulated by glucose, respectively (Figure 4; see Supplemental Table 2 online). Nearly all types of genes were affected by glucose, ranging from stress responses and cellular metabolism to those involved in signaling/gene regulation. Possible gene functions were determined using a variety of methods, including searching gene ontologies (Rhee et al., 2003; Bard and Rhee, 2004; Camon et al., 2004; Harris et al., 2004), conducting pathway analyses (Mueller et al., 2003), and searching the literature. Our results are consistent with the findings from a recent microarray study showing that sugar regulates a broad range of gene types (Thum et al., 2004). Unlike nitrogen regulation, glucose regulation was relatively independent of nitrogen status; however, we cannot rule out a potential role for nitrogen in regulating these genes under different conditions.

Transcriptional Upregulation by Glucose Largely Requires de Novo Protein Synthesis

To confirm the results of the microarray analysis, we conducted RNA gel blot analyses and RT-PCR with a sampling of genes.

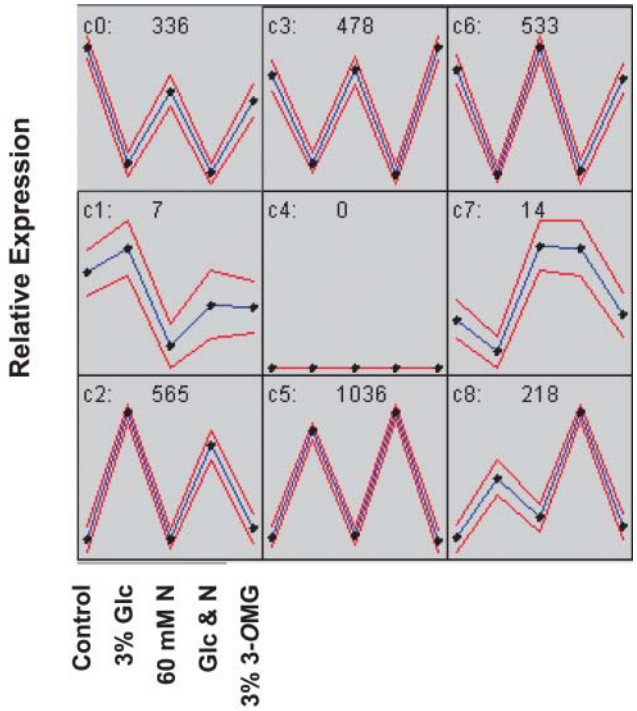

Figure 2. Regulation of Gene Expression Orchestrated by Glucose and Nitrogen.

Cluster analysis was conducted using GeneCluster2 (Golub et al., 1999) using the genes identified in Figure 1, except those showing significant regulation by 3-OMG were removed from consideration. A self-organizing map (SOM) was generated for genes showing greater than a twofold change with expression above background/noise levels. Blue lines represent the mean expression, and the area between red lines represents the range of values within the cluster. This SOM explained 95.1% of the variance occurring in the data set. Value associated with each cluster represents the number of genes with similar behavior.

RNA gel blot analyses were generally effective from genes having signal score greater than 1000, whereas genes with lower expression levels required RT-PCR for detection. The RNA gel blot/RT-PCR analyses were conducted using two sets of RNA from identically prepared plant material except that one set was treated with the protein synthesis inhibitor cycloheximide (CHX) 1 h before the 3-h pulse treatment. In plant material not treated with CHX, results from the RNA gel blot/RT-PCR consistently concurred with the results obtained from the microarray analyses (Figure 3). Some genes had enhanced expression in the presence of CHX compared with those not CHX treated (Figure 3); this is consistent with prior observations of enhanced mRNA stability upon CHX treatment (Baker and Liggit, 1993; Goda et al., 2002). The relative stabilization of some transcripts upon CHX treatment indirectly suggests that posttranscriptional modifications may be occurring. Curiously, CHX treatment did not appear to affect glucose repression; but CHX clearly diminished glucose induction (Figures 3A and 3B), even in cases where CHX stabilized transcript levels. Interestingly, hexokinase 1 and hexokinase 2, dual functional enzymes involving in sugar signaling (Jang et al., 1997; Moore et al., 2003), were no longer induced when CHX was present (Figure 3B). This suggests that glucose repression may not require de novo protein synthesis, but glucose induction appears largely to be a multistep response requiring de novo protein synthesis.

To determine whether CHX treatment disrupts glucose induction on a global scale, microarray analyses were conducted with CHX using the same plant material used for the RNA gel blot analysis. Two independent biological replicates were conducted for each experimental condition containing added CHX; each CHX replicate set was grown concurrently with a set of the non-CHX treated plants used for GeneChip analysis. The FDRs for plants treated with CHX were similar to those without CHX (see Supplemental Figures 3A to 3D online). We were primarily interested in determining how CHX affected expression of the genes regulated without CHX by glucose, nitrogen, or glucose and nitrogen, so CHX data were appended to the non-CHX data described in Figure 2, and genes showing similar expression patterns for both CHX and non-CHX treatments were identified using SOM analysis software (Golub et al., 1999). As shown in Figure 5, only 18% of glucose-inducible genes remained inducible in the presence of CHX; in contrast with glucose induction, 64% of glucose repressible genes were relatively unaffected by CHX. These results suggest that on a global scale, glucose induction is a multistep event requiring de novo protein synthesis, whereas glucose repression occurs to a large extent without de novo protein synthesis. To further analyze the effect of CHX on gene expression, we examined 85 carbohydrate metabolism–related genes out of the 978 glucose-regulated genes described in Figures 4A and 4B (Figure 6; see Supplemental Table 3 online). For a portion of glucose-inducible genes, addition of CHX reduced overall expression to near background levels, thus they could not be meaningfully analyzed by threefold filtering. On the other hand, most of the repressible genes were above background levels, revealing remarkably similar expression profiles for both CHX-free and CHX-treated plants (Figure 6). These results mirror the findings from the RNA gel blot analysis (Figure 3), indicating that the repressive effect of glucose upon transcription remains intact even when de novo protein synthesis is blocked. When the results from Figures 3 and 5 are considered together, they consistently indicate that transcriptional repression by glucose is relatively unaffected by CHX treatment. It isn't totally certain whether the loss of glucose induction upon CHX treatment is because of a direct effect on glucose regulatory mechanisms or a global reduction of expression level; however, the relative stabilization of glucose-repressed transcripts by CHX (Figures 3 and 5) suggests that the former alternative may be more likely.

Transcription Factors Are Differentially Regulated by Glucose

Although sugars are known to have a broad effect on gene expression, it is still intriguing that a large number of transcription factors (TFs) were glucose regulated. Eighty-two glucose-responsive TFs were identified using a threefold filtering criterion; interestingly, a majority of them was downregulated. A similar trend was found when a twofold filter was applied (Figure 7A). Of the TFs identified, most were relatively unaffected by nitrogen (data not shown). Glucose affected 22 families of TFs, including

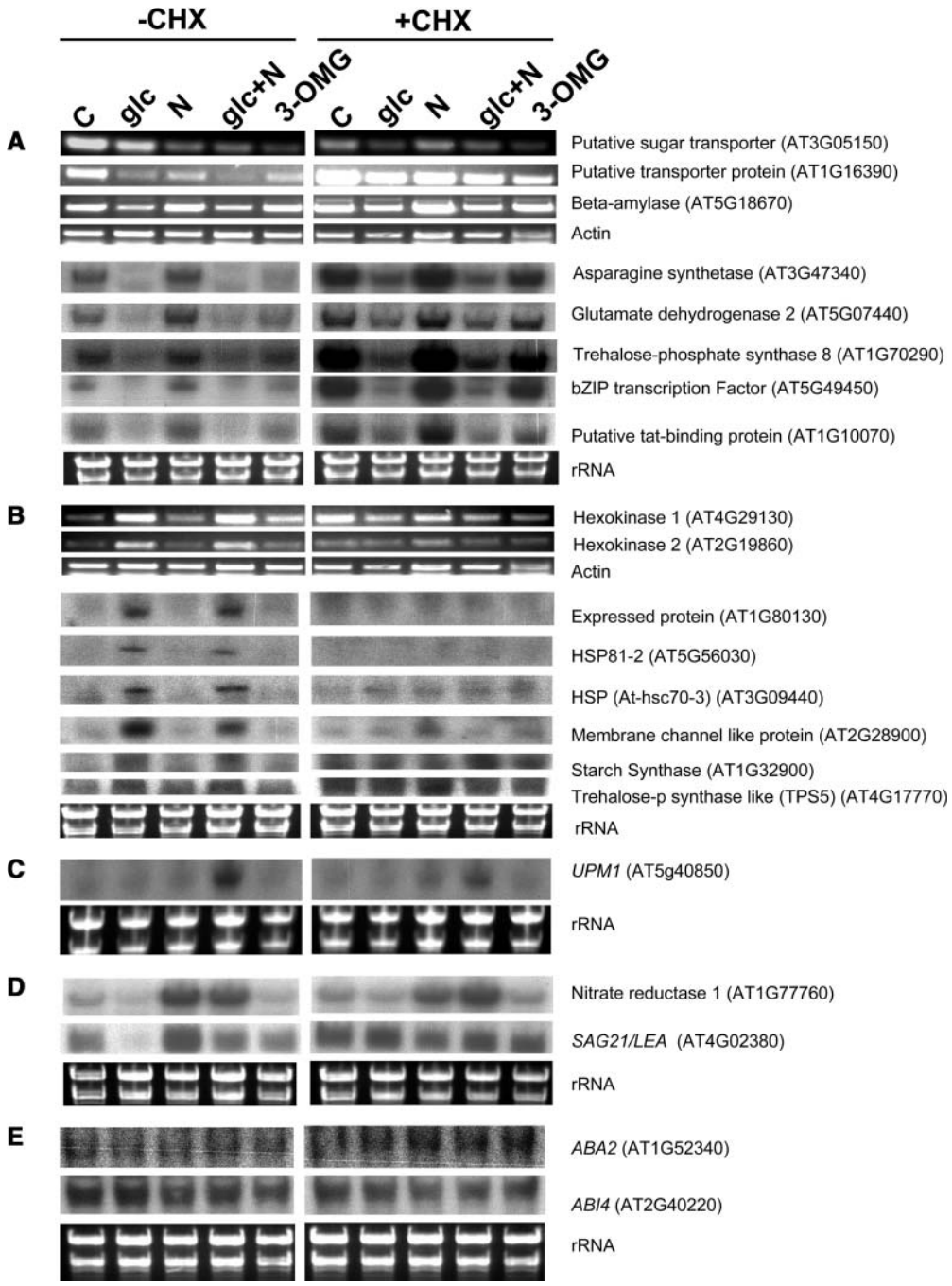

Figure 3. Microarray Data Validation by RNA Gel Blot and RT-PCR Analyses.

Genes chosen for analysis include glucose downregulated genes **(A)**, glucose upregulated genes **(B)**, a gene upregulated specifically by glucose and nitrogen **(C)**, nitrate upregulated genes **(D)**, and two unregulated genes **(E)**.

bHLH, MYB, AP2, and various zinc finger–containing factors. Glucose-regulated TFs account for 8.3% (82/978) of all glucose-regulated genes; this represents relative enrichment of TF in glucose response because TFs are estimated to account for 5 to 7% of the Arabidopsis genome (Riechmann and Ratcliffe, 2000; Jiao et al., 2003). When glucose-regulated TFs are compared with the population of TFs in the Arabidopsis genome (Riechmann et al., 2000; Jiao et al., 2003), factors involved with stress responses (such as some AP2/ERF proteins) appear to be overrepresented upon glucose treatment; by contrast, relatively few developmental factors (such as MADS) appear to be glucose regulated (Figure 7B). Like other genes (Figures 5 and 6),

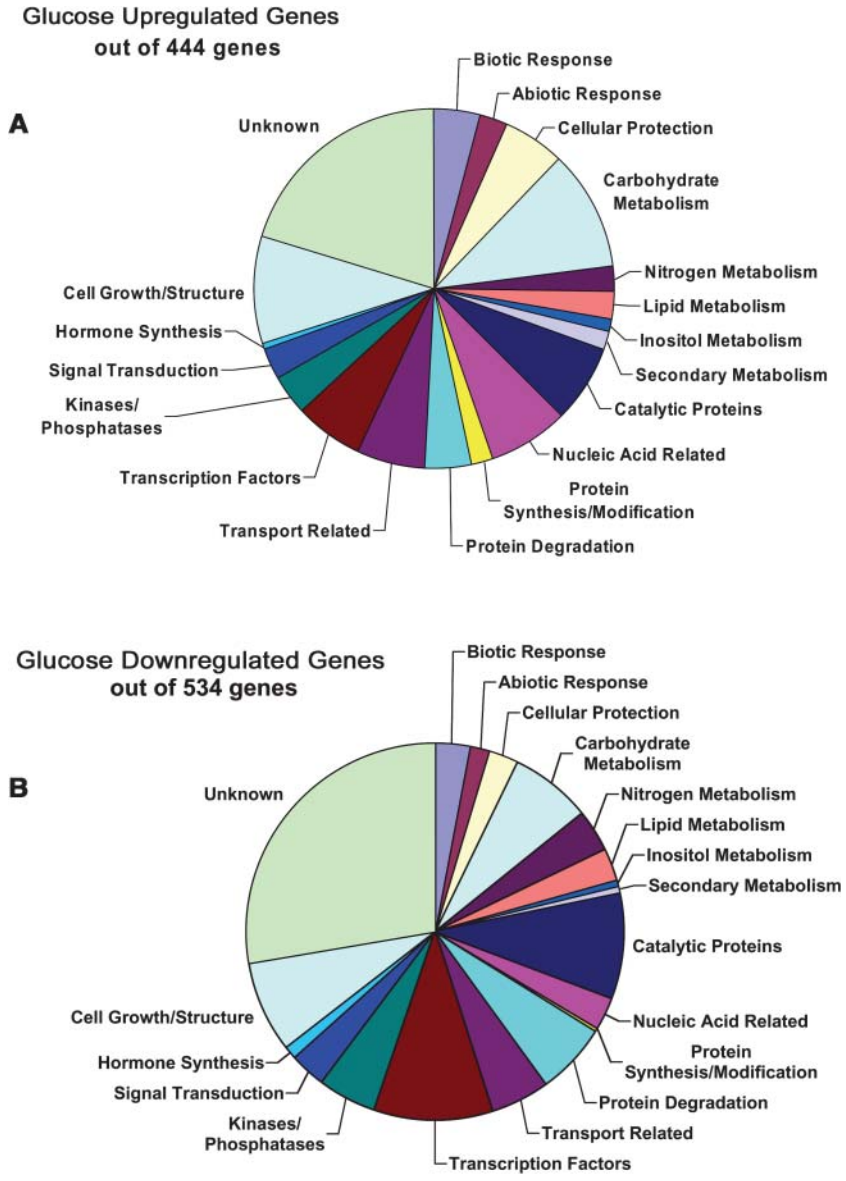

Figure 4. Glucose Regulates Genes with Diverse Functions.

Shown are genes responding to glucose with at least threefold change after normalizing data and conducting RCBD analysis at $P \leq 0.001$. Putative functions were determined using spot annotations (The Arabidopsis Information Resource; http://arabidopsis.org), gene ontology searches (http://www.geneontology.org), pathway analyses, and literature review.

the transcription of TFs was also affected by CHX; whereas up to 95% of the glucose induction was abolished while glucose repression was eliminated to a lesser extent (64%).

Is Sugar-Hormone Cross Talk Mediated through the Change of Hormone Biosynthesis and Perception?

A variety of genetic screens have repeatedly identified genes involved in ABA biosynthesis or response or ethylene perception as being critical for sugar signaling. Loss-of-function of *ABA1* (Arenas-Huertero et al., 2000), *ABA2* (Arenas-Huertero et al., 2000; Cheng et al., 2002), *ABA3* (Arenas-Huertero et al., 2000), *ABI4* (Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000; Rook et al., 2001; Arroyo et al., 2003), *ABI5* (Arenas-Huertero et al., 2000; Brocard et al., 2002; Arroyo et al., 2003; Brocard-Gifford et al., 2003), and *ABI8* (Brocard-Gifford et al., 2004) causes tolerance to developmental stresses caused by exogenous sugar. A considerable amount of genetic evidence also supports an interaction between sugar and ethylene signaling pathways (Zhou et al., 1998; Gazzarrini and McCourt, 2001; Gibson et al., 2001; Rolland et al., 2002; León and Sheen, 2003). Whereas *ctr1* is less sensitive to high concentration of glucose

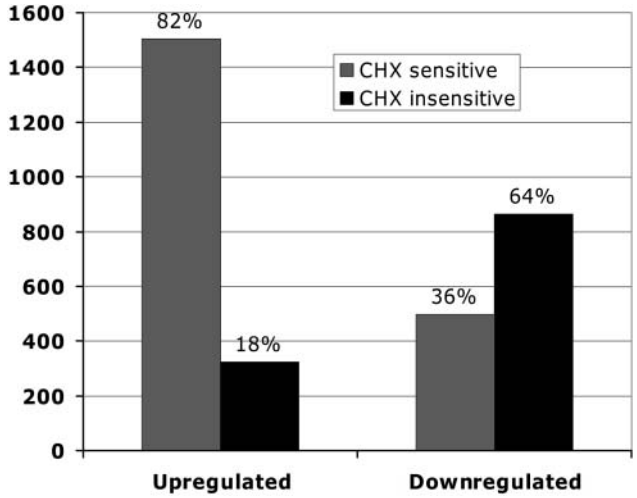

Figure 5. Glucose Induction Often Requires de Novo Protein Synthesis.

Frequency of glucose induction versus glucose repression in the presence of CHX. Expression patterns with and without CHX were determined for the genes identified in Figure 2 using SOM software.

during early seedling development, *etr1* and *ein2* show enhanced developmental arrest caused by sugar (Zhou et al., 1998; Gibson et al., 2001; Cheng et al., 2002; Arroyo et al., 2003; Price et al., 2003). These studies raise a possibility that genes involved in sugar-hormone cross talk might be transcriptionally regulated by glucose. Using a twofold filtering criterion, none of the ABA-related genes previously associated with sugar sensitivity were selected as being glucose-regulated under our conditions (Figure 8A; see Supplemental Table 4A online). However, several ethylene biosynthetic and signaling genes were repressed by glucose, including *CTR1* (Figure 8A) and genes associated with 1-aminocyclopropane-1-carboxylate metabolism (Figure 8B; see Supplemental Table 4B online). Notably, three ethylene biosynthetic genes were downregulated approximately twofold by glucose in the presence of CHX (Table 2). Interestingly, two critical TFs involved in ethylene signaling, *EIN3* and *EIL1*, were also repressed by glucose (Table 2). Together, these results suggest that the transcriptional repression of ethylene biosynthesis and perception may be an early event during glucose signaling. Many of the same ethylene-related genes were recently found to be induced upon prolonged light deprivation without exogenous sugar (Thimm et al., 2004). Because the degradation of EIN3 and EIL1 protein is also enhanced by glucose (Yanagisawa et al., 2003), glucose signaling is likely to affect the ethylene response at multiple levels.

Only a few other hormone biosynthetic genes with an unknown role in plant sugar response were significantly (threefold) regulated by glucose. The ABA biosynthetic gene 9-*cis*-epoxycarotenoid dioxygenase (*NCED1*) (Seo and Koshiba, 2002) was upregulated by glucose (Figure 8B), although *NCED1* also responded to the osmotic control 3-OMG. Nitrilase 3, which is involved in indole-3-acetic acid biosynthesis (Kobayashi et al., 1993), was more specifically upregulated by glucose, whereas two genes associated with jasmonic acid biosynthesis, allene oxide synthase (Simpson and Gardner, 1995) and 12-oxophytodienoate reductase (Schaller et al., 2000), were glucose induced and repressed, respectively. Further studies are needed to determine whether any of these genes are critical components of the sugar response.

Sugar and Stress Response

Another intriguing result is that many stress-related genes are induced by sugar (Figure 8C; see Supplemental Table 4C online). Heat shock proteins are molecular chaperones that assist in the proper conformation of proteins and are strongly upregulated when an organism undergoes a stress (such as heat) that can cause protein misfolding (Larkindale and Knight, 2002). RNA gel blot analysis reveals that cytosolic heat shock protein 70 (*At-hsc70-3*) was clearly upregulated upon glucose or glucose/nitrogen treatment, but regulation of this gene may not be a primary response to glucose because CHX prevented glucose induction of transcription (Figure 3B). The upregulation of an hsp90 is particularly intriguing because hsp90s have been shown to act as buffers in the expression of genes, revealing otherwise hidden phenotypes when hsp90 protein levels become insufficient (Queitsch et al., 2002; Rutherford, 2003). It's possible that the glucose induction of heat shock genes is merely the result of increased metabolic activity. However, other genes associated with stress responses (Knight and Knight, 2001), including those associated with ABA-mediated response, inositol metabolism, and Ca^{2+} regulation, are also largely upregulated by glucose (Figure 8D; see Supplemental Table 4D online). Conceivably, the classic glucose-sensitive phenotype might be partly attributed to a stress response, causing the typical sensitive phenotype except in cases where the normal ABA or ethylene response pathways are altered. The heat shock protein-related stress response is likely to be an indirect event, though, because most of these stress-related genes are no longer regulated by glucose when de novo protein synthesis is blocked.

Transport Proteins Are Largely Regulated According to Function

For many of the functional categories shown in Figure 4, glucose treatment appeared to cause a mixed response, where some genes were upregulated and other genes of similar function were downregulated. One class of glucose-regulated genes where discernable patterns were more evident was the transport proteins. Data in this analysis were filtered after RCBD analysis ($P \leq 0.001$) using a threefold cutoff. Regulation of transport proteins by glucose appeared to be largely determined by function. Other than one gene with glucose phosphate antiporter activity, genes associated with monosaccharide transport, peptide transport, and purine transport were consistently repressed by glucose (Figure 9; see Supplemental Table 5 online). When we analyzed the expression of 13 glucose-responsive monosaccharide transporters—among which sugar transport protein1 (STP1), STP4, STP13, and STP14 were previously identified (Williams et al., 2000)—10 were downregulated at least twofold by glucose, including all four known STPs. Although only STP1 and STP4 are known to be high affinity transporters and STP1

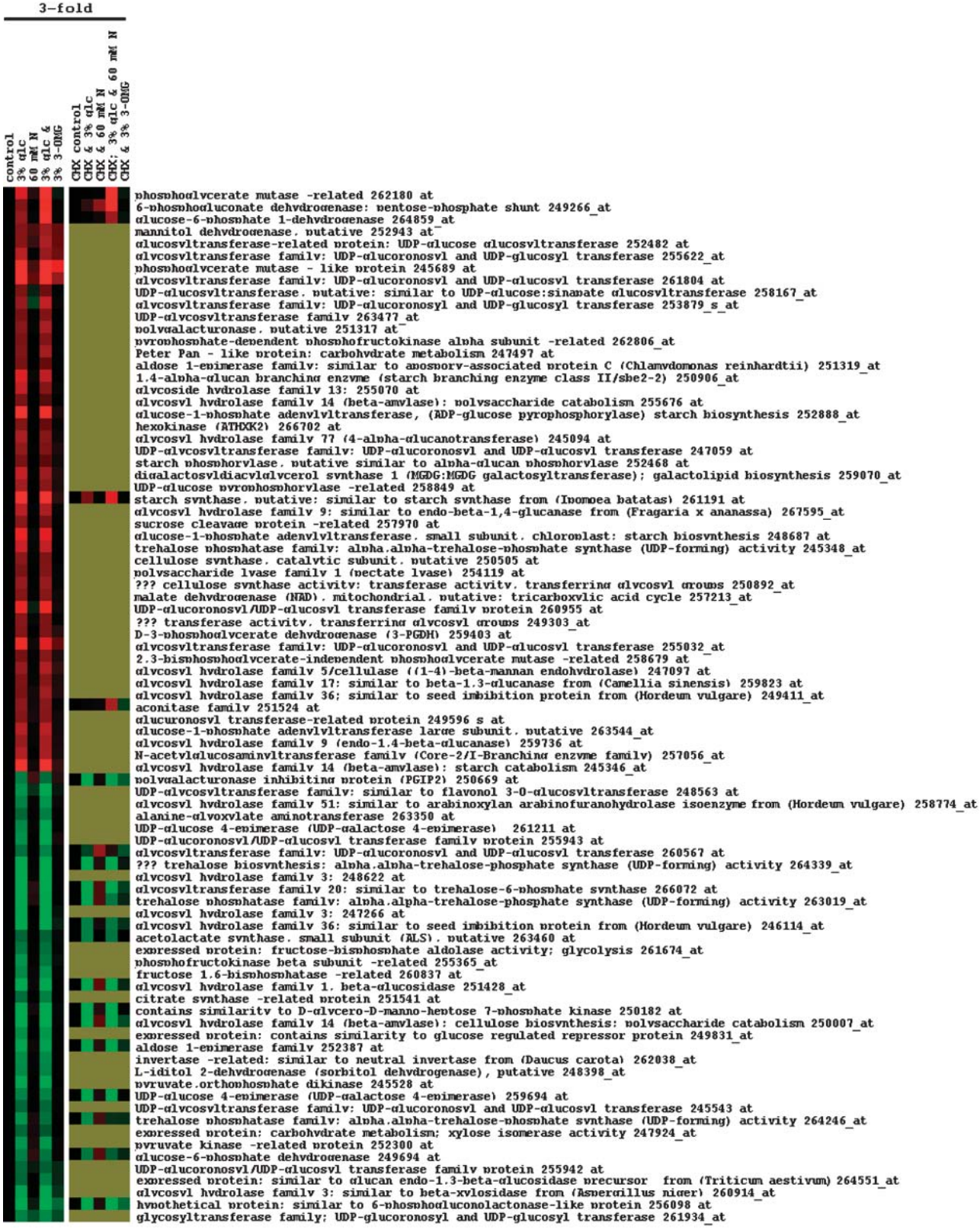

Figure 6. Expression Patterns of Carbohydrate-Related Genes Identified in Figure 4 with or without CHX.

Hierarchical average linkage clustering with correlation measure–based distance (uncentered) was used for the analysis. Red or green represents upregulation or downregulation, respectively, and gray represents either genes at background/noise levels or changes below the fold-change cutoff.

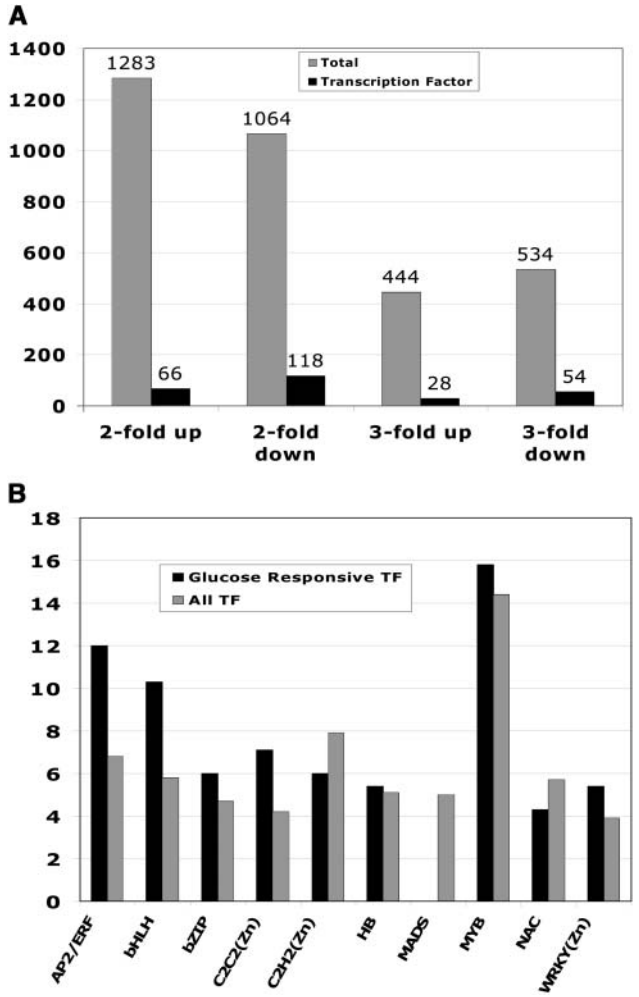

Figure 7. Transcription Factors Are Differentially Regulated by Glucose.

(A) Number of all genes versus transcription factors upregulated or downregulated by glucose with a twofold or threefold cutoff.

(B) Distribution comparison of glucose-regulated transcription factors with all transcription factors in the Arabidopsis genome (Jiao et al., 2003). Percentage of glucose responsive TFs is derived from the number of each category versus total number of glucose responsive TFs.

activity is highly repressed by exogenous glucose (Sherson et al., 2003), the results here raise the possibility that the other eight genes might also be high affinity transporters with a low K_m, where the transcription of these genes may be feedback repressed via the relatively high exogenous glucose level (167 mM). Our findings are consistent with the models in yeast and humans that sugar homeostasis is controlled by sugar transporter activities coupled with sugar-mediated transcriptional regulation (Rolland et al., 2001). Unlike stress-related proteins, glucose repression of monosaccharide transporters is relatively unaffected by CHX (Figure 10). Although more than a dozen of glucose responsive monosaccharide transporters were identified, only one disaccharide transporter, SUC2, showed a modest glucose response in our experiment. This is in agreement with the finding that sucrose-specific signaling pathway was used in transcriptional regulation of sucrose transporter (Choiu and Bush, 1998).

Conversely, genes associated with transporting ions, water, and inorganic metabolites, such as nitrate, phosphate, and sulfate, were generally upregulated upon glucose treatment (Figure 9). The upregulation of these transporters is probably associated with an increase in general metabolism caused by the readily available sugar. This notion is supported by the loss of glucose induction of these genes in the presence of CHX (Figure 9). Other metabolic genes, such as ribosomal proteins, detoxification proteins, and DNA or RNA modifying proteins, were also generally upregulated (data not shown), confirming earlier findings that metabolic activity is increased when sugar becomes available (Thum et al., 2004).

Glucose Regulates Genes Related to Carbohydrate Metabolism

Although Arabidopsis is an oilseed, starch is used in the vegetative stage as a reserve for carbon. Starch synthesis typically requires starch synthase, starch branching enzyme, and glucose-1-phosphate adenylyltransferase (Fernie et al., 2002). Amylases are also involved in starch metabolism. An Arabidopsis isoamylase mutant has been shown to reduce the accumulation of starch while increasing the accumulation of the water-soluble polysaccaride phytoglycogen (Zeeman et al., 1998). Recently, transgenic potato (*Solanum tuberosum*) with antisense expression of Arabidopsis chloroplast-targeted β-amylase has been shown to overaccumulate starch in leaves and reduce starch breakdown during dark treatment (Scheidig et al., 2002). When we treated dark-adapted seedlings with glucose, many of the genes associated with starch biosynthesis were upregulated compared with the carbon-free control (Figure 6). A starch synthase, the 1,4 α-glucan branching enzyme SBE2.2 precursor transcript, three glucose-1-phosphate adenylyltransferase genes (including *APL3*), and an isoamylase-like gene (255070_at) were all significantly upregulated upon glucose treatment. Many of these same genes were shown to be downregulated upon prolonged exposure to darkness in the absence of sugar (Thimm et al., 2004). RNA gel blot analysis confirms induction of a putative starch synthase (Figure 3). Two β-amylases were upregulated, indicating that starch catabolism is also taking place. Collectively, these results suggest that glucose is a critical signal for starch metabolism; this is consistent with the findings of Thum et al. (2004).

Patterns of expression from other genes associated with sugar metabolism reveal a more complex regulatory mechanism. Trehalose has been shown to induce *APL3* expression and thus promote starch synthesis in source tissues; trehalose has also been shown to serve as a stress protection metabolite (Goddijn and van Dun, 1999; Fritzius et al., 2001; Eastmond et al., 2002; Elbein et al., 2003). Trehalose-6-phosphate synthase 1 (TPS1) catalyzes the first step in trehalose biosynthesis (Eastmond et al., 2002). Curiously, we find that one trehalose-6-phosphate synthase-like protein (TPS5, 245348_at) was induced by glucose, whereas three other putative trehalose-6-phosphate synthases, TPS8 (Figures 4 and 7B; 264339_at), TPS9 (263019_at), and TPS10 (264246_at), were strongly repressed by glucose (Figure

Figure 8. Glucose Affects Expression of Ethylene and Stress Associated Genes.

Shown are hierarchical average linkage clustering analyses. Red or green represents upregulation or downregulation, respectively, and gray represents either genes at background/noise levels or no changes with specified cutoff.

(A) Nutrient response of genes implicated in sugar signaling based on genetic studies (León and Sheen, 2003; Gibson, 2004). *ABI4* is not included because expression levels were near background/noise levels. None of these genes showed a more than twofold change in the presence of CHX; however, CTR1, EIN3, and EIL1 were repressed by glucose more than 1.5-fold in the presence of CHX (Table 2).

(B) Nutrient response of hormone biosynthetic genes. Filtering criteria were relaxed to twofold for CHX-treated plants.

6). Whereas other TPS genes were not affected by sugar, the differential regulation of TPS genes by glucose is likely an advantage for adaptation, where differential expression within the same gene family upon a given stimulus results from concurrent spatial- and temporal-specific controls (Eastmond and Graham, 2003). Likewise, a large set of UDP-glucose glucosyltransferases were variably regulated by glucose (Figure 6). UDP-glucose glucosyltransferases are involved in a wide range of functions ranging from regulating phytohormone activity to making macromolecules more soluble (Wetzel and Sandermann, 1994; Jones and Vogt, 2001; Lim et al., 2002). Together, these results suggest that many of the effects glucose has on sugar metabolism do not constitute global responses; rather, the glucose response appears to be targeted to fulfill the specific requirements during growth and development.

Exogenous Glucose Is More Effective Than Nitrogen in Regulating Genes Associated with Nitrogen Metabolism

Sugars and inorganic nitrate are important signaling molecules for adjusting nitrogen and reduced-carbon utilization within a plant (Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001; Forde, 2002; Stitt et al., 2002). Carbon and nitrogen have matrix effects, where genes associated with nitrogen assimilation are upregulated when reduced carbon is abundant and downregulated when reduced carbon is scarce or organic nitrogen is abundant (Coruzzi and Zhou, 2001). When we examined the genes associated with nitrogen assimilation and amino acid metabolism identified in a recent study (Thimm et al., 2004), exogenous glucose appeared to regulate these genes much more profoundly than nitrogen (Figure 11; see Supplemental Table 6 online). Glucose tended to upregulate genes associated with amino acid biosynthesis and downregulate genes related to amino acid catabolism. This concurs with findings from Thimm et al. (2004), where amino acid breakdown was enhanced and biosynthesis was inhibited when plants were exposed to prolonged darkness without exogenously supplied sugar. There were some notable exceptions to the trend we observed. Two glutamate dehydrogenase (*GDH*) genes, which are involved in ammonium utilization and detoxification, and glutamine-dependent asparagine synthetase (*ASN1*), associated with the storage and/or transport of nitrogen from sources to sinks, were both

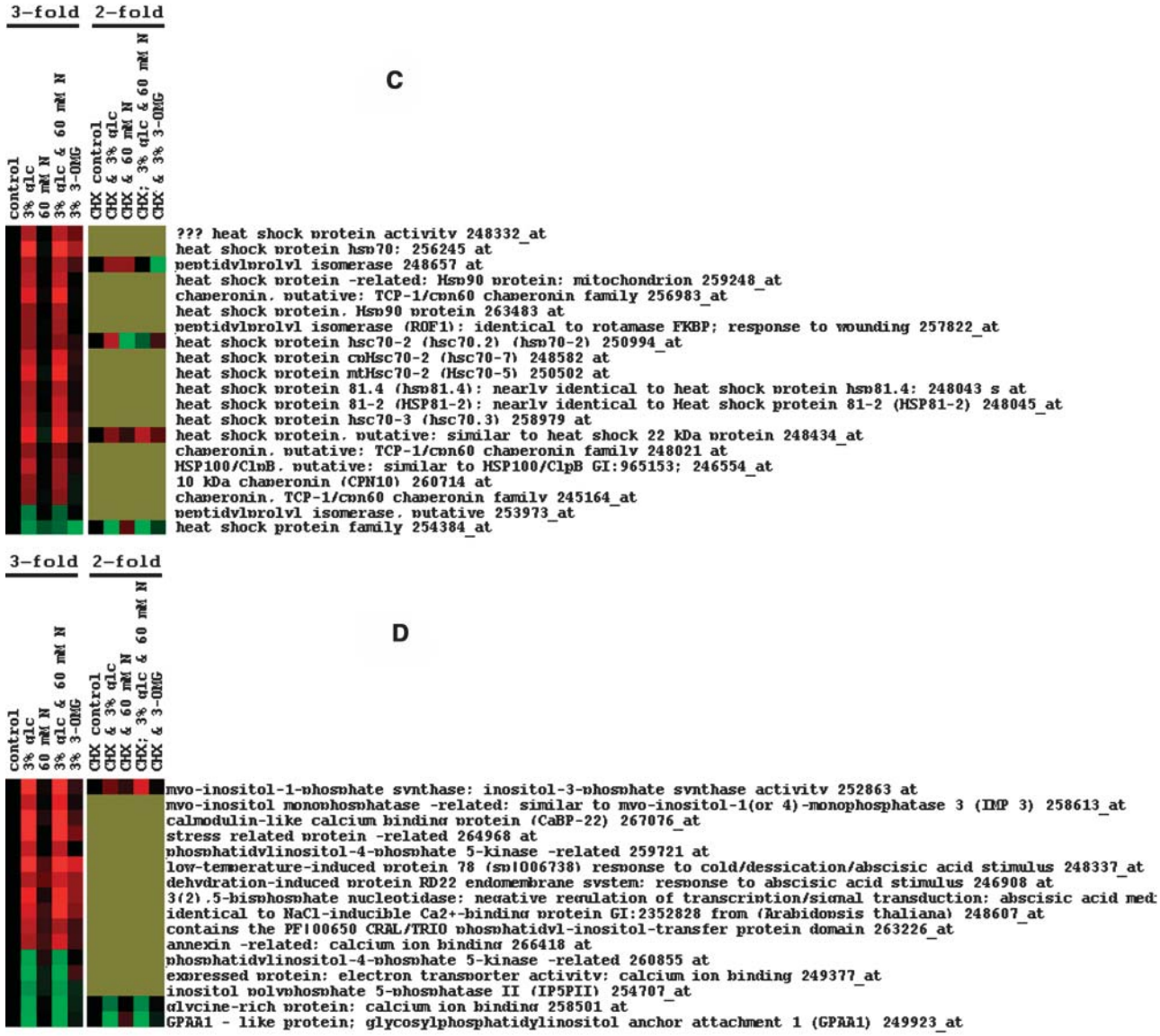

Figure 8. (continued).

(C) Numerous heat shock proteins are affected by glucose.
(D) Other stress-associated genes are highly glucose-responsive.

downregulated by glucose—GDH has been implicated in regulating carbon-nitrogen status (Stitt et al., 2002) and *ASN1* has been previously demonstrated to be tightly regulated by sugars (Lam et al., 1998). RNA gel blot analysis of *ASN1* and *GDH2* confirms the prior observations (Figure 3A). Like putative starch synthase and putative trehalose-6-phosphate synthase (TPS8), *ASN1*, *GDH2*, and tat binding protein (similar to an aminotransferase) have enhanced expression in the presence of CHX (Figure 3A). By contrast, genes associated with assimilation of inorganic nitrate, including NIA1 and ferredoxin-nitrite reductase, were strongly upregulated in the presence of exogenous inorganic nitrogen, even without the presence of exogenous sugars. An earlier study demonstrated that the application of sugars such as sucrose could induce NIA1 expression upon carbohydrate deprivation when nitrate was present (Cheng et al., 1992). Our data show the complementary result, where a nitrogen source is required before a sugar-like glucose can induce NIA1 (Table 1). As predicted by the matrix effect model, the availability of sugar did promote the transcription of most genes involved in nitrogen bioaccumulation in our study. However, for NIA1, exogenous nitrate and sugar are required before induction can occur.

Table 2. The Effects of Glucose on the Expression of Genes Associated with Ethylene Biosynthesis or Signal Transduction

Spot ID	Description	Fold Change CHX: −	Fold Change CHX: +[a]
250911_at	CTR1	−2.5	−1.5
257981_at	EIN3	−2.2	−1.8
266302_at	EIL1	−2.5	−1.7
249125_at	2-Oxoglutarate-dependent dioxygenase, similar to tomato ethylene synthesis regulated protein E8	−3.2	−1.3
247774_at	Oxidoreductase, similar to ACC oxidase	−3.7	−1.9
253999_at	ACC synthase, putative	−3.8	−2.0
246843_at	2-Oxoglutarate-dependent dioxygenase, similar to tomato ethylene synthesis regulated protein E8	−4.3	−3.5
264346_at	ACC oxidase, putative	−3.4	−1.1

[a] Fold-change values for glucose treatment with CHX are compared relative to the CHX control.

DISCUSSION

Global Transcriptional Response to Carbon and Nitrogen in Arabidopsis

This study indicates that glucose affects the transcription of a relatively large proportion of the Arabidopsis genome. Whereas some genes are directly regulated by glucose, others are likely affected indirectly by altered metabolic activities induced by glucose (Figure 12). The scale of the transcriptional change is comparable to the sugar response in other eukaryotes. Depending on the stringency of the filter criteria and the number of genes tested, previous microarray analyses have shown that sugar deprivation significantly alters expression for ~3.8% of the genes in a Drosophila array (fourfold filtering), whereas up to 27% of the genes on a yeast array (twofold filtering) were affected by glucose starvation (DeRisi et al., 1997; Lin et al., 2002; Zinke et al., 2002; Boer et al., 2003). By contrast, the role of inorganic nitrogen is somewhat less pronounced, where although exogenous nitrogen did modify expression of ~8% of the glucose-responsive genes (Figure 3), very few genes were regulated by nitrogen alone. This is consistent with an earlier conclusion that regulation of nitrate reductase in tobacco (*Nicotiana tabacum*) becomes insensitive to nitrate or nitrogen metabolite regulation when sugar levels drop below a certain threshold (Klein et al., 2000). The recent study by Wang et al. (2003) revealed additional nitrogen-regulated genes that were not identified under our conditions. Their study differs from ours in many respects. In particular, their growth medium contained sucrose, and they used a shorter exposure with lower concentration of nitrate. The relatively high concentration of NH_4^+ and NO_3^- used in our experiments may have repressed the expression of some nitrate responsive genes—for instance, NRT2.1, a high-affinity nitrate transporter whose nitrate induction is repressed by NH_4^+ (Gansel et al., 2001; Glass et al., 2002), was not induced under our conditions. Also, the plant material was grown to a different developmental stage and had a longer time period to deplete internally sequestered nitrogen reserves. However, the key difference between their study and ours was that their analysis measured expression changes in shoot and root independently, whereas in our whole plant samples the mass of shoot tissue outweighed root tissue by more than 20-fold. As a result, our whole plant samples behaved remarkably similarly to their shoot tissue. In fact, the differences in gene expression between samples treated with glucose and glucose/nitrogen in our experiment were comparable to the changes seen for their control versus nitrate treatment in shoots because sucrose was used as basal ingredient in all treatments of their experiment (Table 1). It's likely that the glucose response occurs mainly in the shoot and the nitrate response occurs mainly in the root. The higher proportion of glucose-regulated genes seen in our study is probably attributable in part to the overrepresentation of shoot tissue. It's intriguing that a few of the carbohydrate metabolic genes we identified as being glucose regulated were identified in their study as being nitrate regulated in root. Depending on the temporal or spatial conditions, it's possible that both carbon and nitrogen deprivation might regulate genes such as phosphoglycerate mutase (262180_at) and trehalose-6-phosphate synthase (TPS9 and TPS10). Indeed, genes associated with glycolysis and the pentose phosphate pathway have been implicated in nitrogen assimilation and metabolism (Weber and Flugge, 2002; Wang et al., 2003); yet in our study, nitrogen didn't alter expression of any of these genes unless glucose was also present. However, there were considerably more carbohydrate-related genes regulated by glucose, including many associated with starch metabolism, that were unaffected by nitrogen. To date, relatively few genes associated with carbon metabolism have been found to be induced upon addition of nitrate in either Arabidopsis or tomato (*Lycopersicon esculentum*) (Buckhout and Thimm, 2003).

It's possible that the difference in scale between the glucose and inorganic nitrogen responses may be partly attributable to the control mechanisms needed to maintain adequate nutrient levels. When plants are grow in the wild, inorganic nitrogen is usually the nutrient that most limits growth (Forde, 2002). Under normal conditions where plants are not starved for carbohydrate, plants appear to be adapted to assimilate nitrate from available environmental sources (Martin et al., 2002). This is evident in split-root experiments, where a nitrate-treated root half is upregulated for nitrate assimilation compared with a nitrate-deprived root half even when the treatments for both root halves are maintained over a long period (Forde, 2002). Circadian rhythm and the availability of sugar are also known to affect regulation of nitrogen assimilation genes (Cheng et al., 1992; Harmer et al., 2000; Martin et al., 2002). Nevertheless, the degree of coordination necessary for nitrogen assimilation may be relatively simple, so fewer genes would require regulation when an inorganic nitrogen source becomes available. Reduced carbon is a critical starting material for most biosynthesized molecules, and the energy needed to make sugars is available

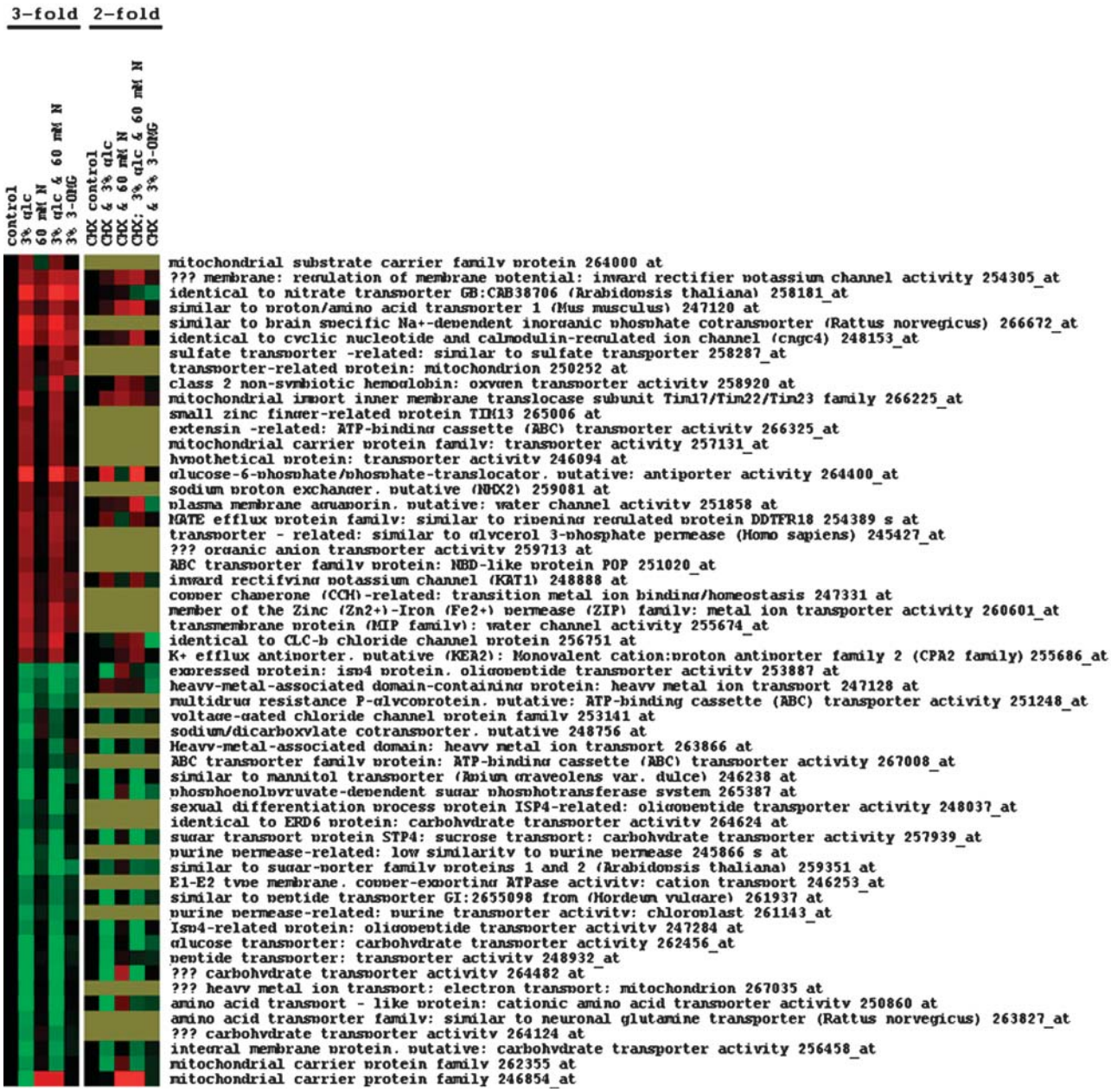

Figure 9. Nutrient Response of Various Transporters.

Shown are hierarchical average linkage clustering analyses. Red or green represents upregulation or downregulation, respectively, and gray represents either genes at background/noise levels or no changes with specified cutoff.

from the environment only during the day when light is present (Winter and Huber, 2000). To maintain adequate levels of reduced carbon as the availability of light varies, plants use newly synthesized sugars when light is present and rely on breakdown of starch reserves during the night (Schleucher et al., 1998). To maintain homeostasis and to take advantage of opportunities when sugar can be made, genes would require precise regulation, coordinating the assimilation of CO_2 as well as the synthesis/mobilization of starch (Huber et al., 1993; Quick, 1996); coordination also would be required to transfer reduced carbon from source to sink tissues (Quick, 1996). Additionally, circadian rhythms affect genes associated with sugar utilization and homeostasis (Harmer et al., 2000), and our data show that once the nitrogen is assimilated, glucose is a key regulator of organic nitrogen metabolism. Consequently, the relative complexity of the response needed to maintain sugar homeostasis may mean that relatively more genes require transcriptional regulation. The difference in complexity for controlling sugar and

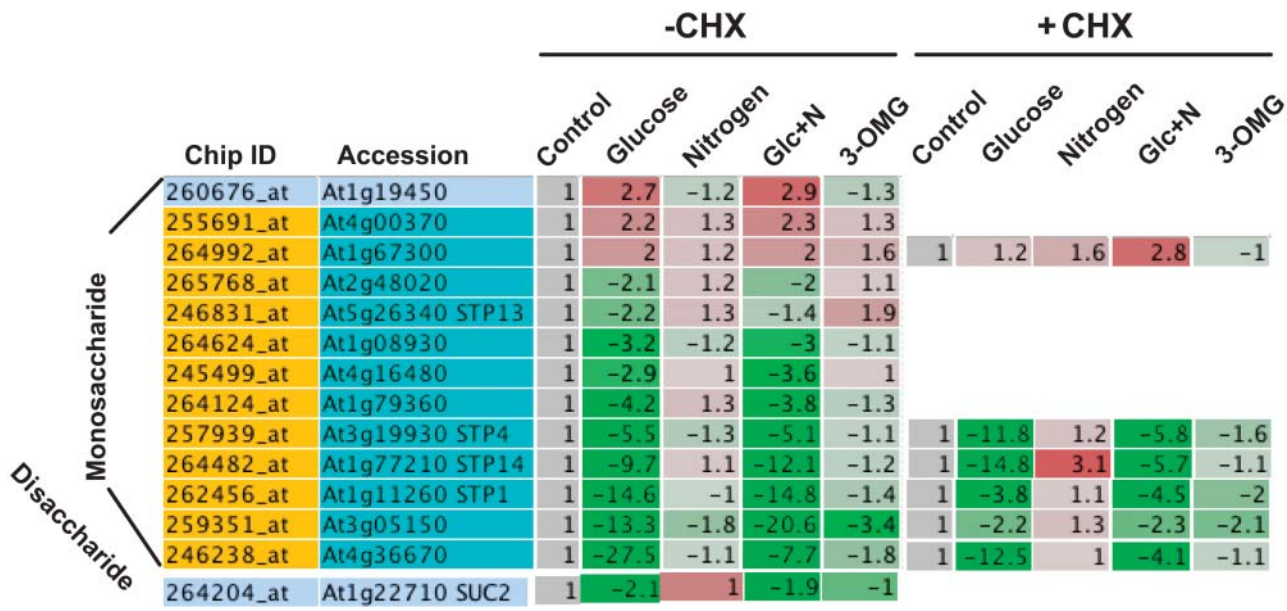

Figure 10. Multiple Sugar Signaling Pathways Revealed by the Regulation of Sugar Transporters.

Glucose has profound effects on the expression of monosaccharide transporters. By contrast, only one disaccharide transporter is affected by glucose, consistent with the idea that disaccharide transporters are uniquely regulated by disaccharides (Choiu and Bush, 1998).

nitrogen levels may provide a partial explanation why a greater proportion of the genome was identified as being glucose regulated rather than nitrogen regulated when we examined global expression patterns. However, this snapshot observation may not fully represent the true response to either nutrient. Plants may respond differently to exogenous and endogenous supplies of sugar, and it's possible that the full response to inorganic nitrogen may not be fully observed within a 3-h timeframe. Alternatively, organic nitrogen sources might be more effective signals in gene regulation; in this study, it isn't clear to what extent the applied inorganic nitrogen is being assimilated to organic forms. A time-course experiment with detailed metabolite profiling would provide insights in the full effects of nitrogen provision. Gene expression profiling using mutants with elevated endogenous sugar levels may address whether exogenously supplied and internally assimilated sugar cause distinct signaling events.

Differential Regulation of Glucose Induction versus Glucose Repression

One unexpected finding was that distinct regulatory mechanisms appear to be controlling transcript abundance when comparing glucose upregulated and downregulated genes. Although transcript abundance can potentially result from post-transcriptional modification (Chan and Yu, 1998a, 1998b; Lam et al., 1998; Cheng et al., 1999), it is likely that some of the expression differences seen upon glucose treatment are the result of transcriptional regulation. Gene transcription is either positively or negatively regulated via the action of transcriptional activators or repressors, respectively. Both types of control proteins are typically modular, where a DNA binding domain typically tethers the regulator to the promoter DNA, whereas a functional domain causes the actual activation or repression of the gene (Ptashne and Gann, 2002). Activators typically function through the recruitment of histone-modifying and -remodeling activities, the direct contact of the regulator with components of general transcription machinery, and the interaction of the transcriptional complex with other coactivators; by contrast, transcription repressors antagonize many of these functions (Workman and Kingston, 1998; King and Kingston, 2001; Ptashne and Gann, 2002). Our results indicate that glucose affects gene transcription via two different mechanisms. The first mechanism is controlled by a process where de novo protein synthesis is not required (CHX insensitive): this is the mechanism used predominantly in glucose repression. The second mechanism, which is blocked by CHX, affects some glucose repressible genes and a large portion of glucose inducible genes. This suggests that glucose induction in plants requires multiple steps, presumably caused by the change of metabolic activities. Loss of glucose response caused by CHX may be because of the inhibition of signaling component, transcription factor, or coactivator biosynthesis, which is required for the induction/repression of certain glucose responsive genes. This possibility may be verified by linking upstream transcription factors with the *cis*-regulatory elements of downstream targets using an approach such as chromatin immunoprecipitation coupled GeneChip analysis (Horak and Snyder, 2002; Lee et al., 2002). There is a precedent in yeast where glucose initially regulates activators through transcriptional repression without requiring de novo protein synthesis (Johnston, 1999; Rolland et al., 2001, 2002; Schuller, 2003). Glucose

Figure 11. Genes Associated with Nitrogen Metabolism Are Predominantly Regulated by Glucose.

The selected genes were normalized and subjected to RCBD analysis ($P \leq 0.001$) and showed a more than twofold transcriptional change.

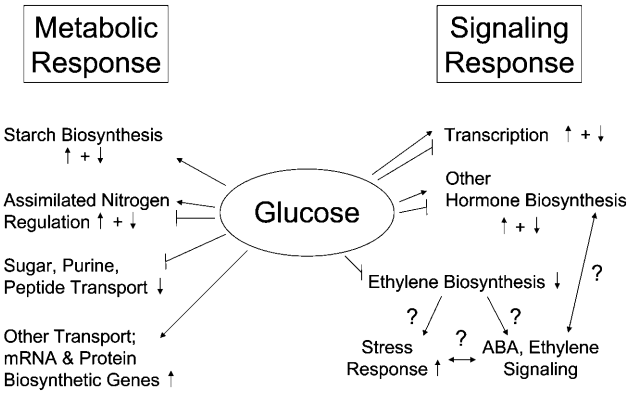

Figure 12. A Proposed Model Summarizes the Metabolic and Signaling Roles of Glucose.

Arrows pointing upward are induction and those pointing downward are repression.

initially activates the yeast Mig1 repression complex via the inhibition of Snf1 kinase activity (Rolland et al., 2002; Schuller, 2003). Mig1 transcription factor recruits corepressors Ssn6 and Tup1 to form a complex, which in turn represses a diverse array of genes including several gene family–specific transcriptional activators involved in alternative carbon usage. The repression of these activators leads to a profound repression of downstream genes whose expression is dependent on these activators. In our study, a majority of transcription factors is repressed by glucose; this raises the possibility that similar glucose repression mechanisms may be conserved in plants. Curiously, a recent microarray study found that sugar- or light-regulated transcription factors were underrepresented compared with the total number of TFs present on the microarray (Thum et al., 2004). This is contrary to our results, where expression of TFs was enriched upon glucose treatment. There are many possible explanations for the difference—1% sucrose rather than 3% glucose was used in their experiments, their sugar treatment time was 8 h as opposed to our 3-h treatment, their plant material was harvested at a later developmental stage, and the results from their 8000-gene microarray chip may not be representative of the full TF response.

Cross Talk between Ethylene, ABA, and Sugar Signaling Pathways

The ethylene signal is transmitted via a pathway that includes a transcriptional cascade, and EIN3 has been identified as a critical component within this cascade (Guo and Ecker, 2004). Recent studies have shown that ethylene enhances the stability of EIN3 and EIL1 proteins (Guo and Ecker, 2003; Potuschak et al., 2003), whereas sugar reduces the stability of these two transcription factors (Yanagisawa et al., 2003). The concerted regulation of EIN3 and EIL1 by ethylene and sugar indicates that cross talk exists between the two signaling pathways. Remarkably, we have found that the transcription of EIN3, EIL1, and CTR1 is also downregulated by glucose (Figure 8A). Consistent with our findings, reduced transcription of CTR1, a mitogen-activated kinase kinase kinase upstream of ethylene signaling transcriptional cascade, was observed in seedlings treated with 7% glucose for 3 h (Arroyo et al., 2003). Our data show that genes associated with ethylene biosynthesis are also transcriptionally repressed by glucose and that repression of three of these genes occurred in the presence of CHX. This raises the possibility that the cross talk between the glucose and ethylene signal transduction pathways may occur through the sugar-mediated transcriptional control of ethylene biosynthetic genes. Earlier findings are consistent with this possibility. Wild-type seedlings were developmentally repressed when grown on MS plates containing 6% glucose, but when seedlings were supplied with ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) in addition to the MS and 6% glucose, the glucose repression was relieved (Zhou et al., 1998; León and Sheen, 2003). Presumably, glucose repression of ACC oxidase (247774_at), ACC synthase (253999_at), and 2-oxoglutarate-dependent dioxygenase (246843_at) may reduce effective endogenous ethylene levels. Because ethylene decreases the sensitivity of seedlings to ABA (Beaudoin et al., 2000; Ghassemian et al., 2000; Gazzarrini and McCourt, 2001) and ABA represses germination and seedling development (Price et al., 2003), a decrease in ethylene caused by glucose may be the key mechanism by which glucose signaling interacts with ABA/ethylene signaling. Further experiments would be needed to confirm this premise.

By contrast, expression of ABA-related genes previously associated with glucose responsiveness was not altered by the conditions used in our study. There are several possible explanations for this observation. Previous experiments have shown that mRNA expression of *ABA2* and *ABI4* does not increase in the presence of 167 mM glucose until germination has occurred (Price et al., 2003). This suggests that the developmental program of the plant can potentially override the effect of sugar for these genes. Also, expression changes in *ABA2* and *ABI4* may constitute an indirect response and thus may not be evident upon a 3-h sugar exposure. In a time-course experiment using 7% glucose, *ABI4* induction in seedlings was shown to begin primarily after 6 h of glucose exposure (Arroyo et al., 2003). A third possibility is that glucose regulation of ABA-related genes may not initially occur at the transcriptional level.

Is Glucose-Induced Stress Response a Physiological Process?

We were also intrigued by how glucose affected transcription of genes associated with stress. Previous studies have demonstrated that high concentration of exogenous glucose stunts the growth of young seedlings (Zhou et al., 1998; Gibson, 2000)—our data raise the possibility that glucose causes a stress response. The actual cellular concentration of glucose resulting from the treatment (167 mM exogenous glucose) is likely to be higher than that typically seen in vegetative plant tissue (Borisjuk et al., 2002)—perhaps there's a threshold where once a certain concentration is reached, a stress response is triggered. The stress response glucose elicits cannot be solely attributed to an osmotic event because 167 mM 3-OMG did not

activate the same stress responsive genes regulated by glucose (Figure 8). The glucose stress response is also distinct from a heat stress response because light has been shown to be essential to observe a phenotypic change with heated plants (Larkindale and Knight, 2002), whereas glucose causes transcriptional and phenotypic changes without light being present (Jang et al., 1997). In any event, a glucose-induced stress response may provide an additional link for the cross talk between sugar signaling and ABA and ethylene signaling. It is known that ABA regulates plant responses when imposed with environmental stresses (Zeevaart and Creelman, 1988). However, additional experiments are needed before a linkage between ABA- and ethylene-signaling events and the glucose stress response seen here can be confirmed. Preliminary results from a microarray study indicate that some of the stress responses seen with glucose treatment are not replicated in seedlings treated with exogenous ABA (J. Price and J.-C. Jang, unpublished results). This is consistent with an earlier study showing that exogenous glucose treatment causes different signaling events than exogenous ABA treatment during germination (Price et al., 2003). However, these results don't rule out a linkage between glucose-induced stress response and ABA. In fact, it is well established that sugar can trigger changes in ABA biosynthesis and signaling (Cheng et al., 2002; Rolland et al., 2002; León and Sheen, 2003); thus, many stress responsive genes are likely coregulated by glucose and ABA. Even during germination, the response of germinating seeds to glucose has been shown to be affected by a block in ABA biosynthetic genes (Price et al., 2003). One possible strategy for dissecting the connections between ABA-related genes and a glucose stress response may be to conduct transcriptional analysis using plants having an ABA deficiency mutation such as *aba2*.

Multiple Sugar Signal Transduction Pathways Revealed by Transcriptional Control of Sugar Transporters

Glucose treatment also resulted in differential expression of sugar transporters. Such control is typical in budding yeast, where several hexose transporters are transcriptionally regulated by multiple glucose signaling pathways (Ozcan and Johnston, 1999). In yeast, some hexose transporter-like genes actually function as signaling receptors rather than actual transporters (Ozcan and Johnston, 1999; Rolland et al., 2001); it's conceivable that some putative sugar transporter genes in plants may have similar signaling functions (Lalonde et al., 1999). In yeast, at least 16 of 48 carbohydrate transporter-like genes have demonstrated transport function (Ozcan and Johnston, 1999; Mewes et al., 2002). Among the rest, Snf3 and Rgt2 have been identified as sugar sensors that can bind to glucose but are unable to transport glucose. Upon binding to glucose, the cytosolic C-terminal portions of Snf3 and Rgt2 interact with downstream signaling components, initiating a signaling cascade and ultimately causing the activation of hexose transporters. This glucose mediated transcriptional regulation controls sugar uptake in yeast (Ozcan et al., 1996, 1998; Ozcan and Johnston, 1999; Ozcan, 2002). In plants, at least 59 sugar and monosaccaride transporters have been putatively identified (Rolland et al., 2001; Mewes et al., 2002), of which 13 monosaccaride transporters and one sucrose transporter are regulated at least twofold under our conditions. Some of the plant sugar transporter-like genes are probably involved in the complex cellular functions, including the maintenance of a balanced source and sink relationship and the regulation of turgor in guard cells (Lalonde et al., 1999; Smeekens, 2000; Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001; Truernit, 2001; Stadler et al., 2003). Whereas sugar transporter-like genes in plants might play more diverse roles than yeast counterparts, the possibility remains that some plant sugar transporter-like genes can act as sugar sensors resembling Snf3 or Rgt2. Although not regulated by glucose under our conditions, AtSut2 (or Suc3) has an extended intracellular domain structurally similar to Snf3 and Rgt2 (Barker et al., 2000).

In summary, our analysis revealed that glucose affected a broad range of genes not previously identified through traditional methods. Besides serving as a critical signal in assessing the general metabolic status, glucose elicits a broad stress response and significantly changes many regulatory genes, including numerous transcription factors. Under the conditions used, nitrogen appeared to have a relatively limited effect on transcriptional patterns, primarily altering expression of genes associated with nitrate assimilation. Much of the regulation for nitrogen utilization appears to be dependent on the availability of reduced carbon, for glucose was much more effective in regulating organic nitrogen metabolism. Nevertheless, nitrogen plays a critical role in modulating the effects of glucose on gene expression. This provides a molecular basis for the importance of carbon/nitrogen ratio in the control of plant growth and development. It is interesting to find that even though glucose causes repression or activation of a similar number of genes, glucose repression may be a somewhat more direct signaling event than glucose activation, which requires de novo protein synthesis. More analysis is needed to confirm the direct and indirect events caused by glucose and to identify *cis*-regulatory elements and *trans*-acting factors involved in the transcriptional activation or repression mechanisms. With the clues provided from GeneChip analyses, new avenues of inquiry may eventually dissect how metabolites like glucose and nitrogen regulate different aspects of the plant life cycle.

METHODS

Preparation of Plant Material and RNA Extraction

A pilot study measuring expression of select genes was conducted to determine suitable conditions for monitoring transcriptional changes caused by sugar and/or nitrogen treatment. Because we use whole plants in our experiments and a recent study demonstrated that transcriptional changes upon nitrogen treatment occur primarily in the root (Wang et al., 2003), the mass ratio of shoot:root was measured in 10 replicates of 10 shoots or roots each to determine whether either tissue is overrepresented under the test conditions used in this study. Exogenous sugar can delay the onset of germination; so to avoid problems interpreting results from plant material at different developmental stages, the initial growth conditions were standardized to allow all plants to be at the early seedling stage. *Arabidopsis thaliana* seeds (ecotype Columbia-0) were surface sterilized and water imbibed in the dark for 3 d at 4°C. Seed pools

were transferred to 1× MS basal salt mixture (GIBCO, Invitrogen) with B5 vitamins, 0.05% Mes, pH 5.7, and 58.4 mM sucrose. The plant material was incubated in the dark at 4°C for 3 d to break dormancy and then was transferred to light at 24°C for 5 d. Cultures were shaken at 140 rpm using an orbital platform shaker (New Brunswick Scientific, Edison, NJ) under continuous white light (100 $\mu E\ m^{-2}\ s^{-1}$).

Once the plant material was uniformly germinated, the experimental conditions were applied. Germinated seedlings were washed seven times with sugar- and nitrogen-free MS to remove residual exogenous sugar or nitrogen, and the plant material was kept in the dark for all subsequent steps. The seedlings were placed in 1× MS salt mixture with B5 vitamins and 0.05% Mes, pH 5.7, but without sucrose or NH_4NO_3 and replacing KCl for KNO_3. Cultures were shaken at 140 rpm at 24°C for 24 h and then either sterile water, NH_4NO_3 and KNO_3 solutions (final concentration 20 mM each), or sugar solution (glucose or 3-OMG; final concentration 167 mM) was added to the medium of randomly selected plant cultures. CHX-treated plants were prepared identically except that 100 μM CHX was applied to seedlings 1 h before the addition of sugar and/or nitrogen. The seedling pools were treated for 3 h shaking at 140 rpm, washed with sterile water, and flash frozen in liquid N_2. The pooled plant material was used for RNA extraction to minimize the effects of variation amongst individual plants. RNA was prepared from frozen tissue using the RNeasy kit (Qiagen, Valencia, CA) following the manufacturer's protocol. The RNA was quantified and tested for quality before it was used for subsequent analyses. Four biological replicates for experiments without CHX and two biological replicates for experiments with CHX were performed. The two biological replicates with CHX were conducted simultaneously with two replicates without CHX.

Labeling of RNA Probe and Hybridization to Arabidopsis GeneChip

Labeling and hybridization of RNA were conducted using standard Affymetrix protocols by the University of California, Irvine DNA MicroArray Facility. Briefly, ATH1 Arabidopsis GeneChips (Affymetrix, Santa Clara, CA) were used for measuring changes in gene expression levels. Total RNA was converted into cDNA, which was in turn used to synthesize biotinylated cRNA. The cRNA was fragmented into smaller pieces and then was hybridized to the GeneChips. After hybridization, the chips were automatically washed and stained with streptavidin phycoerythrin using a fluidics station. The chips were scanned by the GeneArray scanner by measuring light emitted at 570 nm when excited with 488-nm wavelength light. Data from the chips were compiled using MicroArray Suite 5.0 software.

Analysis of GeneChip Data

Data from the GeneChip experiments were analyzed using MicroArray Suite 5.0 and DataMining Tool software as well as Vizard/EPCLUST (Moseyko and Feldman, 2002), GeneCluster2 (Golub et al., 1999), Q (Storey and Tibshirani, 2003), and Cluster/TreeView (Eisen et al., 1998). The MicroArray Suite 5.0 signal was the basis for all subsequent analyses. Two or four independent biological replicates were conducted with or without CHX, respectively. Control versus control scatter plots were generated to assess if the data were linearly distributed. MicroArray Suite 5.0 was used to conduct scalar normalization of the data—because the data consistently appeared to have a linear distribution after normalization with MicroArray Suite 5.0 (see Supplemental Figure 1B online), we did not employ other normalization methods typically used for nonlinear data (Bolstad et al., 2003). To identify significantly regulated genes, the $\log_{10}$ normalized signals were subjected to RCBD analysis using a cutoff of $P \leq 0.001$. The estimated false positive rate was determined using the P value, whereas the estimated FDR was estimated using the software Q (Storey and Tibshirani, 2003). The averaged filtered data were then subjected to an additional filter, which selected genes with greater than a twofold or threefold change versus the control. Threefold change filtering was selected for most analyses. Filtering criteria were primarily selected to minimize the number of false positives and to reduce the number of housekeeping genes considered, many of which are regulated twofold to threefold. Admittedly, the stringent threefold filtering will create some false negatives, as is seen with AtHXK1 (Figure 3); however, relaxing the criterion to twofold will increase the likelihood of including false positives in the data. For nitrogen metabolic genes and CHX regulated genes, filtering was relaxed to twofold change to create a more comprehensive list of regulated genes. To eliminate background noise and reduce false positives, a stringent minimum expression level difference of 140 was set. Probe sets scored as present ranged from 49.7 to 65.4% of the total probe sets. Maximal background for all chips had a signal of 112, whereas maximal raw noise was 6.9 or less.

Confirmation of Transcription Levels with RNA Gel Blot Analysis

The same batches of total RNA used in the microarray experiments were used to conduct RNA gel blot analyses on select genes of interest. RNA gel blot analysis was performed with 5 μg of total RNA/lane using standard protocols (Xiao et al., 2000). The probes for the blots were generated with PCR in the presence of radiolabeled $dAT^{32}P$. For transcripts that were undetectable by RNA gel blot analysis, RT-PCR was conducted using the one-step RNA PCR kit following the manufacturer's protocol (Takara, Madison, WI). The primer pairs used and the accession numbers for both RNA probes and RT-PCR are as follows: transporter protein (At1g16390, 262730_at) forward 5′-AACCCACACGTTTCAAT-3′, reverse 5′-AAACCCAAATGCTCGTT-3′; sugar transporter (At3g05150, 259351_at) forward 5′-ATTTCATCGGTCGGAAA-3′, reverse 5′-TGCAACGCAATTTCA-3′; glycosyl hydrolase (At5g18670, 250007_at) forward 5′-AGACGGTGAGCTGAAA-3′, reverse 5′-TGCCTGAACCTATGCT-3′; ASN1 (At3g47340, 252415_at) forward 5′-TTGCTCACTTGTACGAG-3′, reverse 5′-ATTGCTTAGCCGCCTTA-3′; glutamate dehydrogenase (At5g07440, 250580_at) forward 5′-TGGGCACTAACGCTCA-3′, reverse 5′-CCAAGAGCGCATGGAA-3′; trehalose phosphate synthase 8 (At1g70290, 264339_at) forward 5′-CCCAAGCTTGCTAATATATAGT-3′, reverse 5′-CGGGATCCGACGCGTGGAAGAGTT-3′; bZIP (At5g49450, 248606_at) forward 5′-GGCAAACGCAGAGAA-3′, reverse 5′-AGGACGCCATTGGTTG-3′; tat binding (At1g10070, 264524_at) forward 5′-TCCCCGCGGTACATGTATACATATGCTTAG-3′, reverse 5′-CGGGATCCGTAATCAGCTGGATTTAG-3′; hexokinase 1 (At4g29130, 253705_at) forward 5′-ATGCACAACGACACTT-3′, reverse 5′-TCAGAACTCCAGTGAA-3′; hexokinase 2 (At2g19860, 266702_at), forward 5′-ATGGGTAAAGTGGCAGTTGCAA-3′, reverse 5′-AATTGAACAAAGTCTCAGTAGAAG-3′; expressed protein (At1g80130, 262050_at) forward 5′-GTGGGATGTGGATGAGG-3′, reverse 5′-CTCGATAGGACATGGGT-3′; hsp81-2 (At5g56030, 248045_at) forward 5′-TACGGCTGGACTGCAA-3′, reverse 5′-GAATCAGTCTCTTGAGC-3′; hsp(At-hsc70-3) (At3g09440, 258979_at) forward 5′-CGAGAAGCTTGCTGGAG-3′, reverse 5′-GCTCATCGAAACAAGCG-3′; membrane channel (At2g28900, 266225_at) forward 5′-TGGCAGTGGACATGGG-3′, reverse 5′-AAGCGCGCCACCAAGA-3′; starch synthase (At1g32900, 261191_at) forward 5′-GGCAACTGTGACTGCT-3′, reverse 5′-GCAGCCTGACACAACA-3′; trehalose phosphate synthase 5 (At4g17770, 245348_at) forward 5′-ATGCTCCTTCTTCCGT-3′, reverse 5′-ATCAGCGTTGAGGAGT-3′; NIA1 (At1g77760, 259681_at); SAG21/LEA (At4g02380, 255479_at), forward 5′-CGGGATCCATGGCTTTAAAACATATGCA-3′, reverse 5′-CCATCGATCGCAGCTGCCTTGATTCT-3′; UPM1, (At5g40850, 249325_at) forward 5′-TCCTCCAGTATTCGGA-3′, reverse 5′-CCCTCCTTTCCTTGAAT-3′; ABA2 (AT1g52340, 259669_at) forward 5′-AAAGTGGCATTGATCACT-3′, reverse 5′-TCCTAGTCAAGCCTAGA-3′; ABI4 (At2g40220, 263377_at) forward 5′-CACCGACTAATCAACTT-3′, reverse 5′-CATCTGGACCATCTGAT-3′.

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REFERENCES

Arenas-Huertero, F., Arroyo, A., Zhou, L., Sheen, J., and Leon, P. (2000). Analysis of Arabidopsis glucose insensitive mutants, gin5 and gin6, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. Genes Dev. **14,** 2085–2096.

Arroyo, A., Bossi, F., Finkelstein, R.R., and Leon, P. (2003). Three genes that affect sugar sensing (abscisic acid insensitive 4, abscisic acid insensitive 5, and constitutive triple response 1) are differentially regulated by glucose in Arabidopsis. Plant Physiol. **133,** 231–242.

Baker, E.J., and Liggit, P. (1993). Accelerated poly(A) loss and mRNA stabilization are independent effects of protein synthesis inhibition on alpha-tubulin mRNA in Chlamydomonas. Nucleic Acids Res. **21,** 2237–2246.

Bard, J.B., and Rhee, S.Y. (2004). Ontologies in biology: Design, applications and future challenges. Nat. Rev Genet **5,** 213–222.

Barker, L., Kuhn, C., Weise, A., Schulz, A., Gebhardt, C., Hirner, B., Hellmann, H., Schulze, W., Ward, J.M., and Frommer, W.B. (2000). SUT2, a putative sucrose sensor in sieve elements. Plant Cell **12,** 1153–1164.

Beaudoin, N., Serizet, C., Gosti, F., and Giraudat, J. (2000). Interactions between abscisic acid and ethylene signaling cascades. Plant Cell **12,** 1103–1115.

Boer, V.M., de Winde, J.H., Pronk, J.T., and Piper, M.D. (2003). The genome-wide transcriptional responses of *Saccharomyces cerevisiae* grown on glucose in aerobic chemostat cultures limited for carbon, nitrogen, phosphorus, or sulfur. J. Biol. Chem. **278,** 3265–3274.

Bolstad, B.M., Irizarry, R.A., Astrand, M., and Speed, T.P. (2003). A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics **19,** 185–193.

Borisjuk, L., Walenta, S., Rolletschek, H., Mueller-Klieser, W., Wobus, U., and Weber, H. (2002). Spatial analysis of plant metabolism: Sucrose imaging within *Vicia faba* cotyledons reveals specific developmental patterns. Plant J. **29,** 521–530.

Brocard, I.M., Lynch, T.J., and Finkelstein, R.R. (2002). Regulation and role of the Arabidopsis abscisic acid-insensitive 5 gene in abscisic acid, sugar, and stress response. Plant Physiol. **129,** 1533–1543.

Brocard-Gifford, I., Lynch, T.J., Garcia, M.E., Malhotra, B., and Finkelstein, R.R. (2004). The *Arabidopsis thaliana* abscisic acid-insensitive8 locus encodes a novel protein mediating abscisic acid and sugar responses essential for growth. Plant Cell **16,** 406–421.

Brocard-Gifford, I.M., Lynch, T.J., and Finkelstein, R.R. (2003). Regulatory networks in seeds integrating developmental, abscisic acid, sugar, and light signaling. Plant Physiol. **131,** 78–92.

Buckhout, T.J., and Thimm, O. (2003). Insights into metabolism obtained from microarray analysis. Curr. Opin. Plant Biol. **6,** 288–296.

Camon, E., Magrane, M., Barrell, D., Lee, V., Dimmer, E., Maslen, J., Binns, D., Harte, N., Lopez, R., and Apweiler, R. (2004). The gene ontology annotation (GOA) database: Sharing knowledge in Uniprot with gene ontology. Nucleic Acids Res. **32,** D262–D266.

Chan, M.T., and Yu, S.M. (1998a). The 3′ untranslated region of a rice alpha-amylase gene functions as a sugar-dependent mRNA stability determinant. Proc. Natl. Acad. Sci. USA **95,** 6543–6547.

Chan, M.T., and Yu, S.M. (1998b). The 3′ untranslated region of a rice alpha-amylase gene mediates sugar-dependent abundance of mRNA. Plant J. **15,** 685–695.

Cheng, C.L., Acedo, G.N., Cristinsin, M., and Conkling, M.A. (1992). Sucrose mimics the light induction of Arabidopsis nitrate reductase gene transcription. Proc. Natl. Acad. Sci. USA **89,** 1861–1864.

Cheng, W.H., Endo, A., Zhou, L., Penney, J., Chen, H.C., Arroyo, A., Leon, P., Nambara, E., Asami, T., Seo, M., Koshiba, T., and Sheen, J. (2002). A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. Plant Cell **14,** 2723–2743.

Cheng, W.H., Taliercio, E.W., and Chourey, P.S. (1999). Sugars modulate an unusual mode of control of the cell-wall invertase gene (Incw1) through its 3′ untranslated region in a cell suspension culture of maize. Proc. Natl. Acad. Sci. USA **96,** 10512–10517.

Choiu, T.J., and Bush, D.R. (1998). Sucrose is a signal molecule in assimilate partitioning. Proc. Natl. Acad. Sci. USA **95,** 4784–4788.

Cortes, S., Gromova, M., Evrard, A., Roby, C., Heyraud, A., Rolin, D.B., Raymond, P., and Brouquisse, R.M. (2003). In plants, 3-*O*-methylglucose is phosphorylated by hexokinase but not perceived as a sugar. Plant Physiol. **131,** 824–837.

Coruzzi, G., and Bush, D.R. (2001). Nitrogen and carbon nutrient and metabolite signaling in plants. Plant Physiol. **125,** 61–64.

Coruzzi, G.M., and Zhou, L. (2001). Carbon and nitrogen sensing and signaling in plants: Emerging 'matrix effects'. Curr. Opin. Plant Biol. **4,** 247–253.

Cotelle, V., Meek, S.E., Provan, F., Milne, F.C., Morrice, N., and MacKintosh, C. (2000). 14-3-3s regulate global cleavage of their diverse binding partners in sugar-starved Arabidopsis cells. EMBO J. **19,** 2869–2876.

DeRisi, J.L., Iyer, V.R., and Brown, P.O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. Science **278,** 680–686.

Eastmond, P.J., and Graham, I.A. (2003). Trehalose metabolism: A regulatory role for trehalose-6-phosphate? Curr. Opin. Plant Biol. **6,** 231–235.

Eastmond, P.J., van Dijken, A.J., Spielman, M., Kerr, A., Tissier, A.F., Dickinson, H.G., Jones, J.D., Smeekens, S.C., and Graham, I.A. (2002). Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for Arabidopsis embryo maturation. Plant J. **29,** 225–235.

Eisen, M.B., Spellman, P.T., Brown, P.O., and Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA **95,** 14863–14868.

Elbein, A.D., Pan, Y.T., Pastuszak, I., and Carroll, D. (2003). New insights on trehalose: A multifunctional molecule. Glycobiology **13,** 17–27.

Epstein, C.B., and Butow, R.A. (2000). Microarray technology—Enhanced versatility, persistent challenge. Curr. Opin. Biotechnol. **11,** 36–41.

Fernie, A.R., Willmitzer, L., and Trethewey, R.N. (2002). Sucrose to starch: A transition in molecular plant physiology. Trends Plant Sci. **7,** 35–41.

Forde, B.G. (2002). Local and long-range signaling pathways regulating plant responses to nitrate. Annu. Rev. Plant Biol. **53,** 203–224.

Fritzius, T., Aeschbacher, R., Wiemken, A., and Wingler, A. (2001). Induction of ApL3 expression by trehalose complements the starch-deficient Arabidopsis mutant adg2-1 lacking ApL1, the large subunit of ADP-glucose pyrophosphorylase. Plant Physiol. **126,** 883–889.

Gansel, X., Munos, S., Tillard, P., and Gojon, A. (2001). Differential regulation of the NO3- and NH4+ transporter genes AtNrt2.1 and AtAmt1.1 in Arabidopsis: Relation with long-distance and local controls by N status of the plant. Plant J. **26,** 143–155.

Gazzarrini, S., and McCourt, P. (2001). Genetic interactions between ABA, ethylene and sugar signaling pathways. Curr. Opin. Plant Biol. **4,** 387–391.

Ghassemian, M., Nambara, E., Cutler, S., Kawaide, H., Kamiya, Y., and McCourt, P. (2000). Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. Plant Cell **12,** 1117–1126.

Gibson, S.I. (2000). Plant sugar-response pathways. Part of a complex regulatory web. Plant Physiol. **124,** 1532–1539.

Gibson, S.I. (2004). Sugar and phytohormone response pathways: Navigating a signalling network. J. Exp. Bot. **55,** 253–264.

Gibson, S.I., Laby, R.J., and Kim, D. (2001). The sugar-insensitive1 (sis1) mutant of Arabidopsis is allelic to ctr1. Biochem. Biophys. Res. Commun. **280,** 196–203.

Glass, A.D., Britto, D.T., Kaiser, B.N., Kinghorn, J.R., Kronzucker, H.J., Kumar, A., Okamoto, M., Rawat, S., Siddiqi, M.Y., Unkles, S.E., and Vidmar, J.J. (2002). The regulation of nitrate and ammonium transport systems in plants. J. Exp. Bot. **53,** 855–864.

Goda, H., Shimada, Y., Asami, T., Fujioka, S., and Yoshida, S. (2002). Microarray analysis of brassinosteroid-regulated genes in Arabidopsis. Plant Physiol. **130,** 1319–1334.

Goddijn, O.J., and van Dun, K. (1999). Trehalose metabolism in plants. Trends Plant Sci. **4,** 315–319.

Golub, T.R., Slonim, D.K., Tamayo, P., Huard, C., Gaasenbeek, M., Mesirov, J.P., Coller, H., Loh, M.L., Downing, J.R., Caligiuri, M.A., Bloomfield, C.D., and Lander, E.S. (1999). Molecular classification of cancer: Class discovery and class prediction by gene expression monitoring. Science **286,** 531–537.

Guo, H., and Ecker, J.R. (2003). Plant responses to ethylene gas are mediated by SCF(EBF1/EBF2)-dependent proteolysis of EIN3 transcription factor. Cell **115,** 667–677.

Guo, H., and Ecker, J.R. (2004). The ethylene signaling pathway: New insights. Curr. Opin. Plant Biol. **7,** 40–49.

Harmer, S.L., Hogenesch, J.B., Straume, M., Chang, H.S., Han, B., Zhu, T., Wang, X., Kreps, J.A., and Kay, S.A. (2000). Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. Science **290,** 2110–2113.

Harris, M.A., et al. (2004). The gene ontology (GO) database and informatics resource. Nucleic Acids Res. **32,** D258–D261.

Ho, S.-L., Chao, Y.-C., Tong, W.-F., and Yu, S.-M. (2001). Sugar coordinately and differentially regulates growth- and stress-related gene expression via a complex signal transduction network and multiple control mechanisms. Plant Physiol. **125,** 877–890.

Horak, C.E., and Snyder, M. (2002). ChIP-chip: A genomic approach for identifying transcription factor binding sites. Methods Enzymol. **350,** 469–483.

Huber, S.C., Huber, J.L., and Pharr, D.M. (1993). Assimilate partioning and utilization in source and sink tissues. In International Crop Science I, D.R. Buxton, R. Shibles, R.A. Forsbert, B.L. Blad, K.H. Asay, G.M. Paulsen, and R.F. Wilson, eds (Madison, WI: Crop Science Society of America), pp. 789–797.

Huijser, C., Kortstee, A., Pego, J., Weisbeek, P., Wisman, E., and Smeekens, S. (2000). The Arabidopsis SUCROSE UNCOUPLED-6 gene is identical to ABSCISIC ACID INSENSITIVE-4: Involvement of abscisic acid in sugar responses. Plant J. **23,** 577–585.

Jang, J.C., Leon, P., Zhou, L., and Sheen, J. (1997). Hexokinase as a sugar sensor in higher plants. Plant Cell **9,** 5–19.

Jang, J.C., and Sheen, J. (1994). Sugar sensing in higher plants. Plant Cell **6,** 1665–1679.

Jiao, Y., et al. (2003). A genome-wide analysis of blue-light regulation of Arabidopsis transcription factor gene expression during seedling development. Plant Physiol. **133,** 1480–1493.

Johnston, M. (1999). Feasting, fasting and fermenting. Glucose sensing in yeast and other cells. Trends Genet. **15,** 29–33.

Jones, P., and Vogt, T. (2001). Glycosyltransferases in secondary plant metabolism: Tranquilizers and stimulant controllers. Planta **213,** 164–174.

King, I.F., and Kingston, R.E. (2001). Specifying transcription. Nature **414,** 858–861.

Klein, D., Morcuende, R., Stitt, M., and Krapp, A. (2000). Regulation of nitrate reductase expression in leaves by nitrate and nitrogen metabolism is completely overridden when sugars fall below a critical level. Plant Cell Environ. **23,** 863–871.

Knight, H., and Knight, M.R. (2001). Abiotic stress signalling pathways: Specificity and cross-talk. Trends Plant Sci. **6,** 262–267.

Kobayashi, M., Izui, H., Nagasawa, T., and Yamada, H. (1993). Nitrilase in biosynthesis of the plant hormone indole-3-acetic acid from indole-3-acetonitrile: Cloning of the Alcaligenes gene and site-directed mutagenesis of cysteine residues. Proc. Natl. Acad. Sci. USA **90,** 247–251.

Koch, K.E. (1996). Carbohydrate-modulated gene expression in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47,** 509–540.

Laby, R.J., Kincaid, M.S., Kim, D., and Gibson, S.I. (2000). The Arabidopsis sugar-insensitive mutants sis4 and sis5 are defective in abscisic acid synthesis and response. Plant J. **23,** 587–596.

Lalonde, S., Boles, E., Hellmann, H., Barker, L., Patrick, J.W., Frommer, W.B., and Ward, J.M. (1999). The dual function of sugar carriers. Transport and sugar sensing. Plant Cell **11,** 707–726.

Lam, H.M., Hsieh, M.H., and Coruzzi, G. (1998). Reciprocal regulation of distinct asparagine synthetase genes by light and metabolites in *Arabidopsis thaliana*. Plant J. **16,** 345–353.

Larkindale, J., and Knight, M.R. (2002). Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. Plant Physiol. **128,** 682–695.

Lee, T.I., et al. (2002). Transcriptional regulatory networks in *Saccharomyces cerevisiae*. Science **298,** 799–804.

Lejay, L., Tillard, P., Lepetit, M., Olive, F., Filleur, S., Daniel-Vedele, F., and Gojon, A. (1999). Molecular and functional regulation of two NO3- uptake systems by N- and C-status of Arabidopsis plants. Plant J. **18,** 509–519.

León, P., and Sheen, J. (2003). Sugar and hormone connections. Trends Plant Sci. **8,** 110–116.

Lim, E.K., Doucet, C.J., Li, Y., Elias, L., Worrall, D., Spencer, S.P., Ross, J., and Bowles, D.J. (2002). The activity of Arabidopsis glycosyltransferases toward salicylic acid, 4-hydroxybenzoic acid, and other benzoates. J. Biol. Chem. **277,** 586–592.

Lin, S.J., Kaeberlein, M., Andalis, A.A., Sturtz, L.A., Defossez, P.A., Culotta, V.C., Fink, G.R., and Guarente, L. (2002). Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. Nature **418,** 344–348.

Lu, C.A., Ho, T.H., Ho, S.L., and Yu, S.M. (2002). Three novel MYB proteins with one DNA binding repeat mediate sugar and hormone regulation of alpha-amylase gene expression. Plant Cell **14,** 1963–1980.

Marschner, H. (1995). Mineral Nutrition of Higher Plants, 2nd ed. (London, San Diego: Academic Press).

Martin, T., Hellman, H., Schmidt, R., Willmitzer, L., and Frommer, W.B. (1997). Identification of mutants in metabolically regulated gene expression. Plant J. **11,** 53–62.

Martin, T., Oswald, O., and Graham, I.A. (2002). Arabidopsis seedling growth, storage lipid mobilization, and photosynthetic gene expression are regulated by carbon:nitrogen availability. Plant Physiol. **128,** 472–481.

Melo-Oliveira, R., Oliveira, I.C., and Coruzzi, G.M. (1996). Arabidopsis mutant analysis and gene regulation define a nonredundant role for glutamate dehydrogenase in nitrogen assimilation. Proc. Natl. Acad. Sci. USA **93,** 4718–4723.

Mewes, H.W., Frishman, D., Guldener, U., Mannhaupt, G., Mayer, K., Mokrejs, M., Morgenstern, B., Munsterkotter, M., Rudd, S., and Weil, B. (2002). MIPS: A database for genomes and protein sequences. Nucleic Acids Res. **30,** 31–34.

Moore, B., Zhou, L., Rolland, F., Hall, Q., Cheng, W.H., Liu, Y.X., Hwang, I., Jones, T., and Sheen, J. (2003). Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science **300,** 332–336.

Moseyko, N., and Feldman, L.J. (2002). VIZARD: Analysis of Affymetrix Arabidopsis GeneChip data. Bioinformatics **18,** 1264–1265.

Mueller, L.A., Zhang, P., and Rhee, S.Y. (2003). AraCyc: A biochemical pathway database for Arabidopsis. Plant Physiol. **132,** 453–460.

Ozcan, S. (2002). Two different signals regulate repression and induction of gene expression by glucose. J. Biol. Chem. **277,** 46993–46997.

Ozcan, S., Dover, J., and Johnston, M. (1998). Glucose sensing and signaling by two glucose receptors in the yeast *Saccharomyces cerevisiae*. EMBO **17,** 2566–2573.

Ozcan, S., Dover, J., Rosenwald, A.G., Wolfl, S., and Johnston, M. (1996). Two glucose transporters in *Saccharomyces cerevisiae* are glucose sensors that generate a signal for induction of gene expression. Proc. Natl. Acad. Sci. USA **93,** 12428–12432.

Ozcan, S., and Johnston, M. (1999). Function and regulation of yeast hexokinase transporters. Microbiol. Mol. Biol. Rev. **63,** 554–569.

Potuschak, T., Lechner, E., Parmentier, Y., Yanagisawa, S., Grava, S., Koncz, C., and Genschik, P. (2003). EIN3-dependent regulation of plant ethylene hormone signaling by two Arabidopsis F box proteins: EBF1 and EBF2. Cell **115,** 679–689.

Price, J., Li, T., Kang, S., Na, J., and Jang, J.C. (2003). Mechanisms of glucose signaling during germination of *Arabidopsis thaliana*. Plant Physiol. **132,** 1424–1438.

Ptashne, M., and Gann, A. (2002). Genes and Signals. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Queitsch, C., Sangster, T.A., and Lindquist, S. (2002). Hsp90 as a capacitor of phenotypic variation. Nature **417,** 618–624.

Quick, W. (1996). Sucrose metabolism in sources and sinks. In Photoassimilate Distribution in Plants, E. Zamski and A. Schaffer, eds (New York: Marcel Dekker), pp. 115–156.

Rhee, S.Y., et al. (2003). The Arabidopsis Information Resource (TAIR): A model organism database providing a centralized, curated gateway to Arabidopsis biology, research materials and community. Nucleic Acids Res. **31,** 224–228.

Riechmann, J.L., et al. (2000). Arabidopsis transcription factors: Genome-wide comparative analysis among eukaryotes. Science **290,** 2105–2110.

Riechmann, J.L., and Ratcliffe, O.J. (2000). A genomic perspective on plant transcription factors. Curr. Opin. Plant Biol. **3,** 423–434.

Roitsch, T., Bittner, M., and Godt, D.E. (1995). Induction of apoplastic invertase of Chenopodium rubrum by D-glucose and a glucose analog and tissue-specific expression suggest a role in sink-source regulation. Plant Physiol. **108,** 285–294.

Rolland, F., Moore, B., and Sheen, J. (2002). Sugar sensing and signaling in plants. Plant Cell **14** (suppl.), S185–S205.

Rolland, F., Winderickx, J., and Thevelein, J.M. (2001). Glucose-sensing mechanisms in eukaryotic cells. Trends Biochem. Sci. **26,** 310–317.

Rolland, F., Winderickx, J., and Thevelein, J.M. (2002). Glucose-sensing and -signalling mechanisms in yeast. FEMS Yeast Res. **2,** 183–201.

Rook, F., Corke, F., Card, R., Munz, G., Smith, C., and Bevan, M.W. (2001). Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signalling. Plant J. **26,** 421–433.

Rutherford, S.L. (2003). Between genotype and phenotype: Protein chaperones and evolvability. Nat. Rev. Genet. **4,** 263–274.

Schaffer, R., Landgraf, J., Perez-Amador, M., and Wisman, E. (2000). Monitoring genome-wide expression in plants. Curr. Opin. Biotechnol. **11,** 162–167.

Schaller, F., Biesgen, C., Mussig, C., Altmann, T., and Weiler, E.W. (2000). 12-Oxophytodienoate reductase 3 (OPR3) is the isoenzyme involved in jasmonate biosynthesis. Planta **210,** 979–984.

Scheidig, A., Frohlich, A., Schulze, S., Lloyd, J.R., and Kossmann, J. (2002). Downregulation of a chloroplast-targeted beta-amylase leads to a starch-excess phenotype in leaves. Plant J. **30,** 581–591.

Schleucher, J., Vanderveer, P.J., and Sharkey, T.D. (1998). Export of carbon from chloroplasts at night. Plant Physiol. **118,** 1439–1445.

Schuller, H.J. (2003). Transcriptional control of nonfermentative metabolism in the yeast Saccharomyces cerevisiae. Curr. Genet. **43,** 139–160.

Seo, M., and Koshiba, T. (2002). Complex regulation of ABA biosynthesis in plants. Trends Plant Sci. **7,** 41–48.

Shalev, A., Pise-Masison, C.A., Radonovich, M., Hoffmann, S.C., Hirshberg, B., Brady, J.N., and Harlan, D.M. (2002). Oligonucleotide microarray analysis of intact human pancreatic islets: Identification of glucose-responsive genes and a highly regulated TGF beta signaling pathway. Endocrinology **143,** 3695–3698.

Sherson, S.M., Alford, H.L., Forbes, S.M., Wallace, G., and Smith, S.M. (2003). Roles of cell-wall invertases and monosaccharide transporters in the growth and development of Arabidopsis. J. Exp. Bot. **54,** 525–531.

Shieh, G., and Jan, S.-L. (2004). The effectiveness of randomized complete block design. Stat. Neerl. **58,** 111–124.

Simpson, T.D., and Gardner, H.W. (1995). Allene oxide synthase and allene oxide cyclase, enzymes of the jasmonic acid pathway, localized in Glycine max tissues. Plant Physiol. **108,** 199–202.

Smeekens, S. (2000). Sugar-induced signal transduction in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **51,** 49–81.

Stadler, R., Buttner, M., Ache, P., Hedrich, R., Ivashikina, N., Melzer, M., Shearson, S.M., Smith, S.M., and Sauer, N. (2003). Diurnal and light-regulated expression of AtSTP1 in guard cells of Arabidopsis. Plant Physiol. **133,** 528–537.

Stitt, M., Muller, C., Matt, P., Gibon, Y., Carillo, P., Morcuende, R., Scheible, W.R., and Krapp, A. (2002). Steps towards an integrated view of nitrogen metabolism. J. Exp. Bot. **53,** 959–970.

Storey, J.D., and Tibshirani, R. (2003). Statistical significance for genomewide studies. Proc. Natl. Acad. Sci. USA **100,** 9440–9445.

Temple, S., Vance, C., and Gantt, J. (1998). Glutamate synthase and nitrogen assimilation. Trends Plant Sci. **3,** 51–56.

Thimm, O., Blasing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., Selbig, J., Muller, L.A., Rhee, S.Y., and Stitt, M. (2004). MAPMAN: A user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J. **37,** 914–939.

Thum, K.E., Shin, M.J., Palenchar, P.M., Kouranov, A., and Coruzzi, G.M. (2004). Genome-wide investigation of light and carbon signaling interactions in Arabidopsis. Genome Biol. **5,** R10.

Truernit, E. (2001). Plant physiology: The importance of sucrose transporters. Curr. Biol. **11,** R169–R171.

Wang, R., Guegler, K., LaBrie, S.T., and Crawford, N.M. (2000). Genomic analysis of a nutrient response in Arabidopsis reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. Plant Cell **12,** 1491–1509.

Wang, R., Okamoto, M., Xing, X., and Crawford, N.M. (2003). Microarray analysis of the nitrate response in Arabidopsis roots and shoots reveals over 1,000 rapidly responding genes and new linkages to glucose, trehalose-6-phosphate, iron, and sulfate metabolism. Plant Physiol. **132,** 556–567.

Weber, A., and Flugge, U.I. (2002). Interaction of cytosolic and plastidic nitrogen metabolism in plants. J. Exp. Bot. **53,** 865–874.

Wetzel, A., and Sandermann, H., Jr. (1994). Plant biochemistry of xenobiotics: Isolation and characterization of a soybean O-glucosyltransferase of DDT metabolism. Arch. Biochem. Biophys. **314,** 323–328.

Williams, L.E., Lemoine, R., and Sauer, N. (2000). Sugar transporters in higher plants—A diversity of roles and complex regulation. Trends Plant Sci. **5,** 283–290.

Winter, H., and Huber, S.C. (2000). Regulation of sucrose metabolism in higher plants: Localization and regulation of activity of key enzymes. Crit. Rev. Biochem. Mol. Biol. **35,** 253–289.

Wong, H.K., Chan, H.K., Coruzzi, G.M., and Lam, H.M. (2004). Correlation of ASN2 gene expression with ammonium metabolism in Arabidopsis. Plant Physiol. **134,** 332–338.

Workman, J.L., and Kingston, R.E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu. Rev. Biochem. **67,** 545–579.

Xiao, W., Sheen, J., and Jang, J.C. (2000). The role of hexokinase in plant sugar signal transduction and growth and development. Plant Mol. Biol. **44,** 451–461.

Yanagisawa, S., Yoo, S.D., and Sheen, J. (2003). Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. Nature **425,** 521–525.

Yu, S.M. (1999). Cellular and genetic responses of plants to sugar starvation. Plant Physiol. **121,** 687–693.

Yunus, M., Pathre, U., and Mohanty, P. (2000). Probing Photosynthesis: Mechanisms, Regulation, and Adaptation. (London, New York: Taylor & Francis).

Zeeman, S.C., Umemoto, T., Lue, W.L., Au-Yeung, P., Martin, C., Smith, A.M., and Chen, J. (1998). A mutant of Arabidopsis lacking a chloroplastic isoamylase accumulates both starch and phytoglycogen. Plant Cell **10,** 1699–1712.

Zeevaart, J., and Creelman, R. (1988). Metabolism and physiology of abscisic acid. Annu. Rev. Plant Physiol. Plant Mol. Biol. **39,** 439–473.

Zhou, L., Jang, J.C., Jones, T.L., and Sheen, J. (1998). Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. Proc. Natl. Acad. Sci. USA **95,** 10294–10299.

Zhu, T. (2003). Global analysis of gene expression using GeneChip microarrays. Curr. Opin. Plant Biol. **6,** 418–425.

Zinke, I., Schutz, C.S., Katzenberger, J.D., Bauer, M., and Pankratz, M.J. (2002). Nutrient control of gene expression in Drosophila: Microarray analysis of starvation and sugar-dependent response. EMBO J. **21,** 6162–6173.