A plant’s response to gravity is a complex mechanism involving stimulus perception, signal transduction and a differential growth response. Disturbance of any of these steps would affect a plant’s ability to respond to gravity. The gravity persistence signal (gps) mutants were identified using a cold treatment to select for mutants specifically defective in the signal transduction events prior to auxin redistribution. Plants perceive gravity at 4°C but auxin transport is abolished; transport is restored upon return to room temperature (RT) (Wyatt et al. 2002). Although auxin transport is restored in gps1 after return to RT, auxin is not asymmetrically redistributed, and the inflorescence stems fail to bend in response to the cold gravistimulation (Nadella et al., 2006). To identify additional genes in the pathway, especially those affected by the gps1 mutation, we analyzed differential transcript abundance between gps1 and wild-type (WT).

\(\text{gps1}\) and wild-type plants were grown to maturity, gravistimulated at 4°C for 1 h and returned to RT (GPS treatment) (Wyatt et al. 2002). Inflorescence stems were sprayed with RNAlater (Ambion) 5 min after return to RT, and tissue from the region of elongation was collected and analyzed for relative changes in transcript levels. As a control for the cold treatment, additional WT and \(\text{gps1}\) plants were placed vertically at 4°C for 1 h, returned to RT and tissue collected as above. Total RNA was extracted and purified (RNAeasy, Qiagen) from 10-12 plants for each treatment and sent to the Microarray Facility at University of California, Irvine for amplification and hybridization to Arabidopsis ATH1 GeneChips (Affymetrix). Expression values were normalized across each chip using the Cross Gene Error Model (GeneSpring, Silicon Genetics, CA) and significance determined by a Student T-test (p value=0.1).

From the 24,000 transcripts analyzed, 258 genes showed differential expression: 128 up-regulated and 129 down-regulated in \(\text{gps1}\) as compared to wild-type after cold gravistimulation (Fig. 1). Those genes that were similarly expressed in response to the cold treatment alone were eliminated from further consideration.

Ten genes were selected, based on level of differential expression, for analysis of transcript abundance throughout the GPS treatment (Table 1). Both WT and \(\text{gps1}\) plants were grown to maturity, cold gravistimulated for 60 min then returned to vertical at RT (Wyatt et al. 2002). RNA was extracted from the elongation zone of the inflorescence stems at 0, 30, and 60 min after gravistimulation and at 70 min after gravistimulation (10 min after return to vertical at RT). Transcrip abundance was determined by real time PCR using an ABI7900HT sequence detection system according to Kimbrough et al. (2004). Actin8 expression was measured separately for each time point, and the values used to normalize transcript abundance of the selected genes.
Based on expression pattern, most genes fell into two categories. Transcripts of the Spot 3 vacuolar sorting protein, 3-methylcrotonyl-CoA carboxylase and hypothetical protein increased dramatically in WT within 30 min after gravistimulation but were virtually absent in gps1 (Fig. 2) suggesting that these genes may be regulated either directly or indirectly by GPS1. Transcript level of the phospholipase, CONSTANS B box protein, bZIP transcription factor and cyclophilin were either unaffected or down-regulated in WT but spiked in gps1 between 30 and 60 min after gravistimulation (Fig. 3). These genes appear to be negatively regulated by GPS1 or its products.

Although several of these genes are novel to gravitational biology, potential roles for a vacuolar sorting protein or a phospholipase could be imaged. Morita et al. (2002) suggested that vacuole formation and functioning plays a key role in the early stages of shoot gravitropism. Phospholipase A2 has also been implicated in shoot gravitropism of Arabidopsis by auxin mediated cell elongation (Lee et al., 2003). An increase in the At2g39400 transcript in gps1 suggests that a phospholipase, regulated by the GPS1 protein, might be directly or indirectly involved in lateral auxin redistribution thereby affecting cell elongation and contributing to the gps1 no response phenotype. In any event, we have identified genes that appear to be regulated early in the gravity signal transduction pathway and provide interesting new candidates for study.

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