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REGULATION OF TRANSCRIPTION IN ROOTS OF ARABIDOPSIS GRAVITY MUTANTS.

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Plant growth is guided by gravity. The mechanism by which plants sense gravity is not understood. Sedimentation of starch-containing amyloplasts (statolith) in the columella cells is involved in the perception of changes in the orientation towards the vector of gravity. Inositol-1,4,5,-triphosphate ($InsP_3$), Ca^{2+}, and proton transport, as well as phytohormones (auxin, cytokinin, ethylene, brassinosteroids), reactive oxygen species, and nitric oxide have been shown to mediate the gravity induced signal transduction. Several factors involved in gravitropism have been identified by mutants defective in their response to changes in their orientation towards gravity. In order to elucidate the role of some of these signal transduction elements in their regulation of gravity specific transcription, we analyzed temporal changes in gravity specific up-regulated genes in mutant and their respective wildtype backgrounds (ecotypes) after reorientation.

Increases in transcript abundances of some genes occur within 1 minute after reorientation in the *Arabidopsis* root apex, and are regulated specifically by gravity, not mechanical stimulation (Kimbrough et al. 2004). Focusing on three of these gravity-specific genes, we present evidence that gravity-induced transcript abundance changes of these genes are not strictly conserved between ecotypes. In the root gravity response mutants *arg1* (defective in a DNAJ protein; Sedbrook et al. 1999) and *arl2* (defective in DNAJ protein similar to ARG1, Guan et al. 2003), transcript abundance changes in those selected gravity specific genes were not initiated. This indicates that the DNAJ protein is required for gravity-induced transcriptional regulation. In the gravity response mutant *adk1* (defective in adenosine kinase, Young et al., unpublished) transcript abundance of one gene was up-regulated, while two other genes did not show an increase in transcript abundance after reorientation. This indicates that transcriptional regulation of those genes requires different signal transduction elements in response to gravity stimulation.

Methods

We harvested *Arabidopsis* root apices (7 day old etiolated seedlings) in order to analyze their relative changes in transcript level in response to gravistimulation. For our time course experiment, approximately 150 root tips were harvested before (0 time point) and 2, 5, 15 min after 135° reorientation by pouring RNAlater (Ambion) onto the plates and cutting off the root apex (~7.5 mm). Total RNA was extracted, purified with RNeasy (Qiagen), and cDNA was synthesized using gene specific primers. *Arabidopsis* gravity signaling mutants *arg*, *arl,* and *adk* along with wild type controls were grown for seven days in the dark, reoriented (135°) and time-dependent changes in transcript abundance measured for the gravity specific genes (At4g23670; At5g38020; At5g48010).

Results

The gravity-response mutant plants used in this study were derived from the *Arabidopsis* ecotypes Wassilewskja (mutants *arg, arl*) and Landsberg (mutant *adk).* We measured gravity-induced transcript abundance changes in these ecotypes in comparison to Columbia-O which was used in the microarray study (Kimbrough et al. 2004). We found that there are differences in the gravity-induced transcriptional regulation of specific genes between different *Arabidopsis* ecotypes in both temporal profile and magnitude of regulation. (Figures 1 A,B,C).

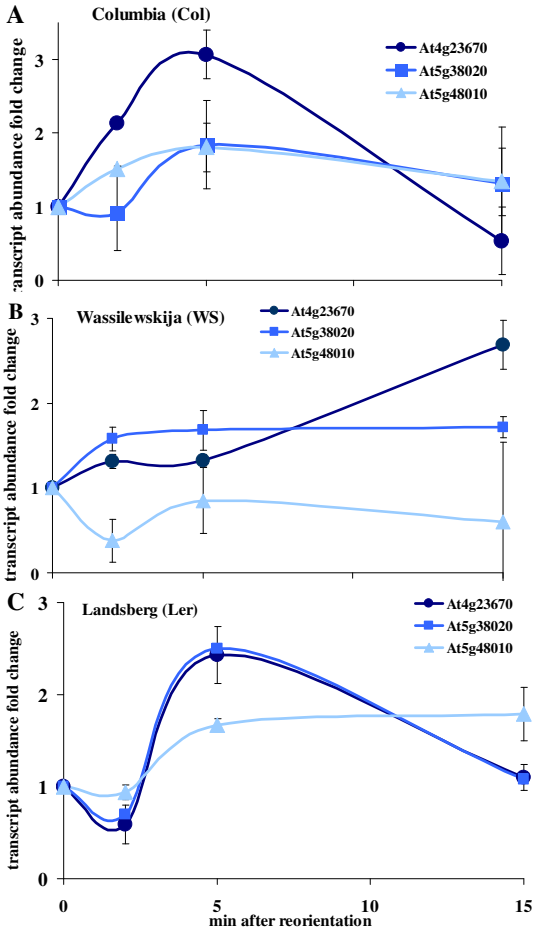

Figure 1: *Transcript abundance changes of At4g23670, At5g38020, and At5g48010 in response to reorientation in Arabiodopsis ecotype Columbia (A), Wassilewskija (B) and Landsberg (C).*

DNAJ protein is essential for gravity induced transcription

ARG and ARL code for DNA-J proteins which are essential for gravity-induced regulation of transcript abundance changes for the genes we tested. Reorientation of these gravity mutants did not induce any significant increases in transcript abundance of the genes tested compared to their respective ecotype (Figures 2 A,B).

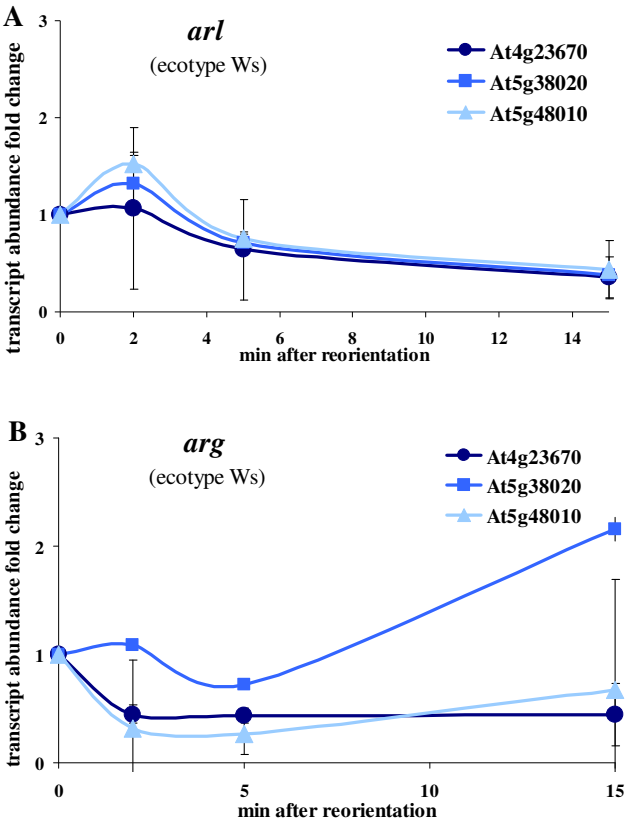

Figure 2: *Transcript abundance changes of At4g23670, At5g38020, and At5g48010 in response to reorientation in Arabiodopsis mutants arl (A), and agr (B)*

Adenosine kinase (ADK) regulates transcription of some genes probably involved in hormone synthesis

ADK is involved in modulating root-tip architecture and gravitropism in the *Arabidopsis* root tip. Mutation in this gene alters gravitropic sensitivity (Masson et al., unpublished). Transcript levels of At5g38020 (SamT-functions in Ado-Met pathway as methylating enzyme) and At5g48010 (AtPEN1 – involved in brassinosteroid synthesis) do not increase in the root apex after gravity stimulation, while transcriptional regulation of At4g23670 (Major latex protein-like) is enhanced or at least not repressed. This indicates that ADK is part of a signal transduction pathway that activates transcriptional regulation for At5g38020 and At5g48010. It is therefore "downstream" of arg and arl in the signal transduction pathway.

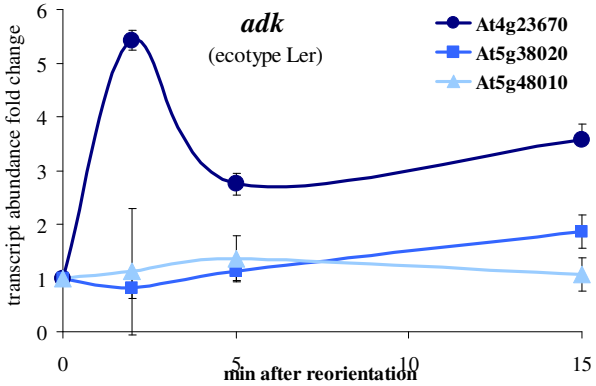

Figure 3: *Transcript abundance changes of At4g23670, At5g38020, and At5g48010 in response to reorientation in Arabiodopsis mutant adk.*

Conclusions

❖We measured variation in the gravity-induced transcriptional regulation of specific genes between different *Arabidopsis* ecotypes.

❖ARG and ARL code for proteins which are essential for gravity-induced regulation of transcript abundance changes for at least those genes tested.

❖ADK expression is required for gravity-induced transcriptional regulation of some specific genes (At5g38020, At5g48010) possibly involved in hormonal regulation.

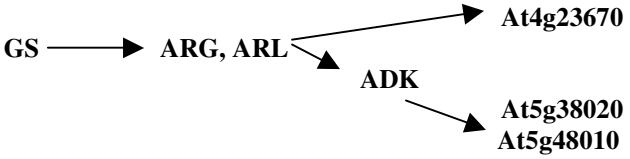

Figure 4: The *transcriptional regulation induced by gravity stimulation (GS) requires the DNAJ proteins ARG and ARL. The transcription of the Major-latex like gene (At4g23670) does not require functional expression of ADK.*

(Supported by NASA grant NAG2-1566 to C.S.B. and North Carolina Space Consortium grant to J.W.Y.)

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