Summary of Research
Molecular Control of Cell Growth During Gravity Responses of Maize Seedlings
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Introduction

Gravity influences plants in many ways via its physical effects on the convective flows of gases and liquids, the buoyancy and sedimentation of cellular organelles, and the distribution of mechanical stresses in weight-bearing structures. These physical effects lead to a variety of reactions and adaptive developmental responses in plants. Perhaps the best-studied plant gravity response is gravitropism - the "homing in" of growing organs towards a particular angle with respect to gravity. Most plants respond to gravity by gravitropic bending of roots downwards and stems upwards. Such gravitropic bending arises from differential cell growth on the two sides of the bending organ. For this project we hypothesized that such growth differences arise from differences in *expansin* activity, which come about because of organ-level asymmetries of H^+ efflux and expansin export to the wall.

Expansins are wall proteins that mediate pH-dependent wall extension. Expansins are encoded by a large superfamily of genes that are divided into two groups, named α-expansins and β-expansins. These genes share about 25% amino acid identity. Our published results indicate that β-expansins act selectively to loosen grass (Type II) cell walls, which differ from dicot (Type-I) walls in the composition of their matrix polysaccharides, whereas α-expansins have greater activity on Type-I walls. Our comparative analysis of the Arabidopsis and rice genomes indicates that the number of β-expansin genes is unusually high in grasses (18 in rice versus 6 in Arabidopsis). Moreover, based on data from microarrays and databases of ESTs (Expressed Sequence Tags), we find that β-expansins are expressed at higher levels in grasses (maize, rice) than in Arabidopsis.

For this project, we proposed a number of experiments to elucidate the role of α- and β-expansins during gravitropic bending of maize coleoptiles and roots. This plant material is chosen because it is a model system for gravitropism studies and because it will permit us to explore the biological functions of β-expansins in grasses (Arabidopsis has a dicot-type wall and is nearly lacking in β-expansins). Our specific goals were:

1. To identify the α- and β-expansin genes expressed in maize coleoptiles and roots;
2. To determine whether expression of these genes is altered by auxin and during gravitropism;

3. To evaluate the relative significance of α- and β-expansins for growth and gravitropic bending;
4. To screen for specific peptide inhibitors of expansins;
5. To identify and characterize mutants in one of more expansin genes involved in gravitropism of the maize coleoptile and root;
6. Additional experiments were added to assess change in the biophysical properties of the cell wall during gravitropism.

These experiments are important for elucidating the molecular basis for differential control of cell growth during gravitropism, and may have wider implications in agriculture, where grasses play a major role in feeding humanity. I provide a concise summary of our results below.

Goal 1

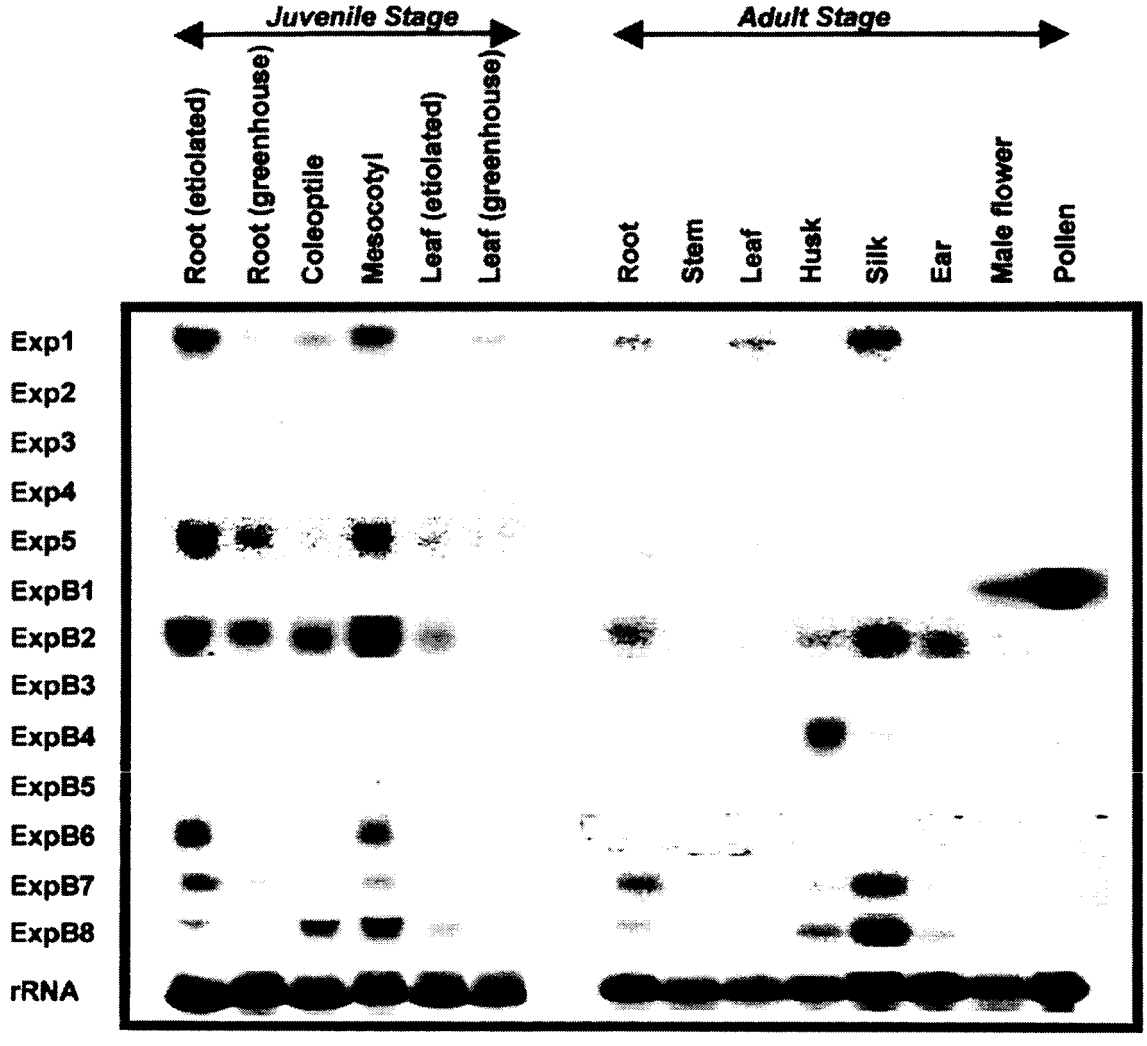
In this work we were assisted by Dr. Robert Meeley of Pioneer HiBred, who made available maize expansin clones from their propriety collection and also carried out initial PCR screens of their maize mutant collection (so-called TUSC technology). In addition we screened a maize root cDNA library and also analyzed the recent maize EST collection in GenBank. By these means we identified and obtained sequences for five a-expansin cDNAs and 8 β-expansin cDNAs. The table below lists some of the characteristics of these 13 cDNAs:

Table 1. *A list of maize expansin cDNAs*

Name (α or β)	cDNA Length	3'-UTR	5'-UTR	Predicted Signal Peptide	Predicted Mature Protein
		bp		*amino acids*	
Exp1, α	~1,160; full	255	61	20	233
Exp2, α	~1,285; full	410	43	22	254
Exp3, α	~1,040; full	167	63	29	233
Exp4, α	~1,029; partial	321	–	–	>235
Exp5, α	~1,040; full	324	9	18	208
ExpB1, β	~1,145; full	220	31	24	245
ExpB2, β	~1,270; full	380	45	24	>285
ExpB3, β	~800; partial	138	–	–	>216
ExpB4, β	~1,279; full	240	100	24	284
ExpB5, β	~660; partial	102	–	–	>176
ExpB6, β	~1,150; full	211	83	26	250
ExpB7, β	~1,170; full	282	64	28	240
ExpB8, β	~1,480; full	520	80	26	260

We designed gene-specific probes for each of these cDNAs and used northern blotting (RNA gel blots) prepared from total RNA of various plant parts to determine the expression pattern of these expansins. The results are shown in figure 1 below. In roots the following genes were expressed: Exp1, Exp5, ExpB2, ExpB6, ExpB7 and ExpB8. In coleoptiles the following genes were expressed: Exp1, ExpB2, ExpB8. In mesocotyls the following genes were expressed: Exp1, Exp5, ExpB2, ExpB6, ExpB7 and ExpB8.

Figure 1 (below): Northern blot analysis of expansin gene expression in various tissues of the maize seedling and mature plant.



Goals #2 & #3

We tried to assess the possibility for differential expression of these expansin genes in the coleoptile and the root using in situ hybridization, tissue-printing, and Northern blot analysis. Dark-grown maize seedlings were turned to a horizontal position and allowed to bend. We monitored the time course of bending with a infra-red sensitive video camera. At the point of maximal rate of curvature, the coleoptile was excised and cut longitudinally to divide top half and bottom half. Similar protocols were developed for root tissue analysis. The tissues were frozen in liquid nitrogen and subsequently extracted for total RNA and analyzed by Northern blotting with gene-specific probes. We found that the bottom half of the coleoptile had slightly higher levels of EXP1 (bottom:top ratio of 1.6; statistically significant at the 5% level).

Is this difference in EXP1 expression large enough to account for the differential growth of the two sides of the coleoptile? No, it could account for at best a minor amount of the differential growth. Other expansin genes did not appear to be to show differential expression at this point in time.

Is this difference in EXP1 expression large enough to be accounted for by auxin redistribution? To answer this we assayed the expression of EXP1 in coleoptile segments +/- exogenous auxin, and compared the results with the gravitropism experiment. EXP1 expression was enhanced by auxin, and based on published reports of auxin redistribution we concluded that the differential expression of EXP1 was consistent with approximately 2:1 redistribution of auxin on the two sides of the coleoptile.

For tissue printing, the root or coleoptile was cut in cross section in the midpoint of the curving region and imprinted onto nylon or nitrocellulose membranes. These were then developed as for a Northern blot with gene specific probes. We could not detect a consistent and reliable asymmetry in expansin expression by this method.

Similar, in situ hybridization did not indicate a consistent and reliable asymmetry in expansin expression.

We concluded, therefore, that at the transcript level, the alteration of expansin expression was small: large enough to be accounted for a auxin redistribution, but not large enough to account for more than a minor change in the growth of the coleoptile.

To test these conclusions in another way, we assayed cell wall mechanical properties with different techniques, to assess whether the walls showed an asymmetry in the characteristic "signature" of expansin action (Goal #6).

Goal #4

Due to technical difficulties (protein availablility), we were not able to identify peptides that bound appropriate expansins with high specificity.

Goal #5

For goal #5 we chose to search for a maize mutant in Exp1 (an α-expansin). We obtained 4 lines from Pioneer HiBred with putative Mu-transposon insertions in Exp1. We cloned and sequenced the DNA flanking the transposon insertion, to confirm the insertion and to identify its specific location. This was successfully done with the 4 lines. However, in subsequent generations the EXP1-flanked Mu disappeared. This indicates a somatic insertion in the first generation of plants, and so it did not pass to subsequent generations.

Goal #6

Expansin has a characteristic effect on the physical properties of cell walls: it increases the stress relaxation of the cell walls, as well as the sustained "creep" of walls (long-term irreversible extension), but it does not have effects on the elastic and plastic compliances,

as tested with the Instron stress/strain analyzer. We tested coleoptile walls for asymmetry (top:bottom) in these physical properties during the time course for coleoptile gravitropism (at 30 min and 75 min after start of stimulation). No consistent or statistically significant difference were found for any of the assays. These results are consistent with the small magnitude of EXP1 mRNA asymmetry.

CONCLUSIONS

Our major conclusion from these experiments is that gravitropic bending of the maize coleoptile is accompanied by only a small change in expansin mRNA distribution, which is not enough to cause the large asymmetry in growth during coleoptile bending. The results are consistent with a redistribution of auxin, and this in turn may alter cell wall pH, which in turn would alter expansin activity without a major change in expansin protein or transcript abundance. This hypothesis is consistent with published reports of apoplastic pH changes during gravitropism, but needs to be evaluated in a quantitative fashion.

Inventions:
None

Related Publications:

Cosgrove, D.J., Link, B.M., and Wagner, E.R. Analysis of expansin expression and cell wall properties in relation to gravitropic bending of maize coleoptiles and roots. *In preparation.*

Cosgrove, D. J. "Wall structure and wall loosening. a look backwards and forwards." Plant Physiol 125.1 (2001): 131-34.

Cosgrove, D. J. et al. "The growing world of expansins." Plant Cell Physiol 43.12 (2002): 1436-44.

Link, B. M., E. R. Wagner, and D. J. Cosgrove. "The effect of a microgravity (space) environment on the expression of expansins from the peg and root tissues of *Cucumis sativus*." Physiol Plant 113.2 (2001): 292-300.

Wu, Y., R. B. Meeley, and D. J. Cosgrove. "Analysis and expression of the alpha-expansin and beta-expansin gene families in maize." Plant Physiol 126.1 (2001): 222-32.