Pursuit - The Journal of Undergraduate Research at the University of Tennessee

Volume 2
Issue 1 *Spring 2011*

Article 8

March 2011

Arabidopsis thaliana Myosin XI is Necessary for Cell Fate Determination in Root Epidermis

Kevin Kuo
University of Tennessee, Knoxville, kkuo@utk.edu

Pursuit: The Journal of Undergraduate Research at the University of Tennessee

Pursuit

Arabidopsis thaliana Myosin XI is Necessary for Cell Fate Determination in Root Epidermis

KEVIN KUO
Advisor: Andreas Nebenführ

Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville

Root hairs are specialized epidermal cells with important functions, such as absorbing water and nutrients from soil. In Arabidopsis thaliana, *root hairs develop in a strict position-dependent mechanism that results in alternating longitudinal hair and non-hair cell files along the surface of the root. This invariant pattern is determined, in large part, by a complex network of transcription factors in the epidermis. However, epidermal cells are also highly plastic and have the ability to promote root hair growth even after the hairless cell fate has been determined by transcriptional control. Recently, it has been observed that the mutants of a specific isoform of class XI myosins,* mya1, *grow ectopic root hairs in* Arabidopsis. *Since class XI myosin proteins are actin-based molecular motors that transport intracellular organelles or protein complexes, it is possible that* MYA1 *also has a role in the trafficking of those transcription factors and their upstream regulators that are involved in cell-type patterning. Here, three promoter-GUS (beta-glucuronidase) constructs were utilized to determine what possible cargo or cargoes MYA1 may carry in this signal-transduction pathway.* GL2::GUS *expression in the roots of 4- to 6-day-old seedlings was first analyzed to determine any differences in patterning between wild-type and mutant genetic backgrounds. The results suggested that the elimination of* MYA1 *did not affect the expression patterning of GL2, which is the output of the signaling pathway.* MYA1, *thus, may not be involved in the transcriptional regulation that determines epidermal cell fate in* Arabidopsis. *It also suggested that* MYA1 *may then act on the hormonal or nutrient starvation response in root hair development to override the default cell fates set by the network of transcription factors.*

Introduction

Root hairs are specialized epidermal cells

The epidermis is the outer layer of cells that help organisms respond to different stimuli from the dynamic external environment, while allowing them to maintain a stable internal environment. It can exist in a wide variety of specific morphologies and functions. Root hairs are classic examples of epidermal cells with a clear shape and purpose. These specialized epidermal cells are tubular outgrowths that extend from roots to provide a greater surface area to absorb water and nutrients (Guimil and Dunand, 2006).

In *Arabidopsis thaliana*, these fine epidermal structures develop according to two independent but related processes: patterning and morphogenesis. The first phase involves the spatial determination of where root hairs develop on the root. In other words, by the end of this stage, epidermal cells will have acquired a distinct identity that defines what they will later become, which results in the patterning of future hair cells and hairless cells. The hair precursor cells are known as trichoblasts, while the non-hair precursor cells are named accordingly as atrichoblasts. Following cell fate determination, these trichoblasts and atrichoblasts then grow and transform into their final shapes in the morphogenesis phase (Guimil and Dunand, 2006). Trichoblasts grow a tubular appendage, while the atrichoblasts only elongate.

Epidermal cell fate in *Arabidopsis* roots is determined by transcription factors

The patterning phase of root hair development in *Arabidopsis* has been studied by developmental biologist for over a decade because of its simplicity in appearance and growth. Clear reporter-gene expression and controlled experimentation are possible due to the roots' transparency and ability to grow on defined media. Additionally, root hairs in *Arabidopsis* develop according to an invariant patterning system that parallels the spatial relationship between epidermal and cortical cells (Schiefelbein et al., 1997). More specifically, developing epidermal cells that situate in the cleft between two cortical cells (the H-cell position) preferentially differentiate into trichoblasts, while those outer cells that overlay one cortical cell (the N-cell position) become atrichoblasts (Figure 1) (Kwak et al., 2005).

This patterning and spatial relationship, in turn, is determined by a set of putative transcriptional regulators (Figure 2). At the start of the pathway, a transmembrane protein SCRAMBLED (SCM) in root epidermal cells detects an unknown positional cue from the cortex and transduces it into a signal that represses the expression of the transcription

Figure 1. A cross-section of an Arabidopsis root. Epidermal cells positioned in the cleft between two cortical cells differentiate into hair (H) cells, while epidermal cells located along single cortical cell walls differentiate into non-hair (N) cells.

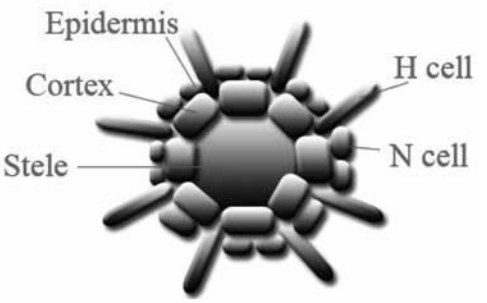
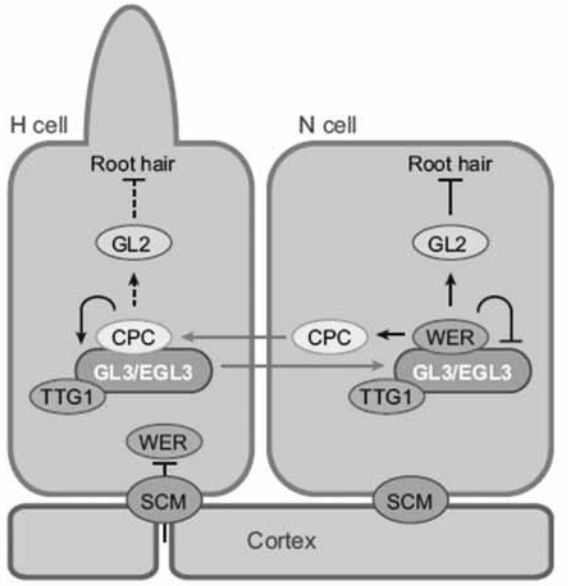


Figure 2. Regulatory model of epidermal cell fate determination in *Arabidopsis roots*. A positional cue from the cortex binds to a receptor-like kinase SCM situated on the epidermal cell membrane. SCM then initiates the transcriptional machinery, which ultimately results in the expression or repression of GL2 depending on the cell position. Diagram reproduced from (Ishida et al., 2008).



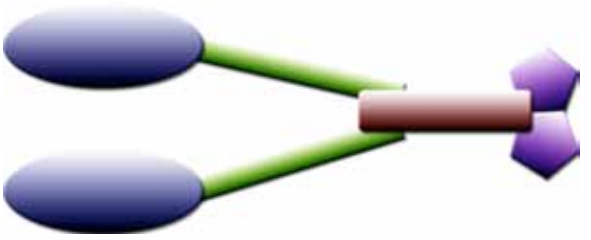
factor WEREWOLF (WER) in future trichoblasts. In future atrichoblasts, WER and ENHANCER OF GLABRA3 (EGL3) form complexes that bind directly to the promoters of *GLABRA2 (GL2)* and *CAPRICE (CPC)*, stimulating the expression of these two transcription factors in N cells. GL2 determines the non-hair cell fate by activating an unknown inhibitory pathway, while CPC moves into the neighboring future trichoblast. In future trichoblasts, CPC competes with WER for EGL3. CPC and EGL3 form complexes that cannot activate GL2 expression and, thus, result in the hair cell fate (Ishida et al., 2008).

Although much of the transcriptional regulatory network that determines epidermal cell-fate is well established, the molecular basis of its initiation, output, and movement of the relevant transcription factors is unknown. The latter is the focus of this research. The reporter constructs of WER, EGL3, and GL2 are particularly useful in understanding cell-fate determination as they demonstrate distinct expression patterns, which can be used in reverse genetic studies.

Class XI myosins may transport transcription factors and their upstream regulators

Myosins are molecular motor proteins in eukaryotes that use ATP as an energy source to move along actin filaments. These proteins consist of three main regions: a motor (N-terminal) domain that provides the power stroke needed to pull against actin filaments, a neck (regulatory) domain that associates with divalent cation binding proteins, and a tail (C-terminal) domain that binds to a diverse array of cargoes (Figure 3) (Ojangu et al., 2007). Although there are at least 34 classes of myosins among various organisms, there are only two classes represented in *Arabidopsis*, class VIII and XI. Within each of these two classes, several isoforms exist as evident from various biochemical, molecular, and genetic studies. The function of each of these isoforms remains largely unknown. However,

Figure 3. A schematic model of class XI myosins. The blue ovoid structures represent the motor domain, while the green rods exemplify the neck domain. The purple section represents the tail domain.



class XI isoforms demonstrate numerous structural similarities with class V isoforms found in fungi and animals. This close structural resemblance suggests that class XI myosins are likely involved in the transport of large intracellular structures — a function that has been putatively attributed to class V myosins (Li and Nebenführ, 2007).

Mutants of a particular isoform of class XI myosins, *mya1*, in *Arabidopsis* have been shown to develop ectopic root hairs (Figure 4) (Park, 2010). This phenotype is especially noteworthy for several reasons. First of all, the spatial arrangement of epidermal transcription factors is critical in regulating cell fate, whether an epidermal cell matures into a future hair cell or non-hair cell. It is possible that ectopic root hair growth occurs when MYA1 is not available to correctly transport a transcriptional regulator to its neighboring epidermal cell through the plasmodesmata (Figure 5A). Another attractive possibility is that MYA1 may have a role in inhibiting SCM's ability to down-regulate *WER* (Figure 5B). SCM has been shown to be preferentially, but not exclusively, expressed in future hair cells (Kwak and Schiefelbein, 2008). MYA1, as a result, may be involved in the transport of a signal molecule or protein complex that directly or indirectly inhibits the repressive effects of the minute levels of SCM expressed in future non-hair cells. This redundancy in regulation of *WER* may have evolved to ensure correct cell patterning in the root epidermis. According to this model, *mya1* mutants may grow more roots hairs because SCM activity in the N cell

Figure 4. Root hair phenotypes. Mutant roots (A) developed more roots hairs than wild-type lines (B) under the same conditions. Images were taken under visible light by a stereomicroscope. Images reproduced from (Park, 2010).

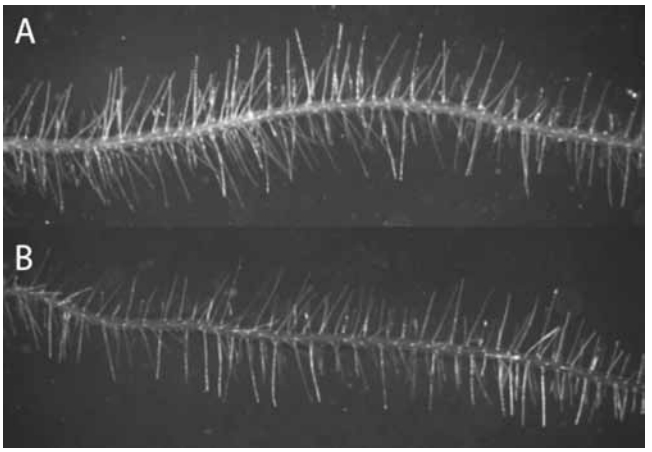
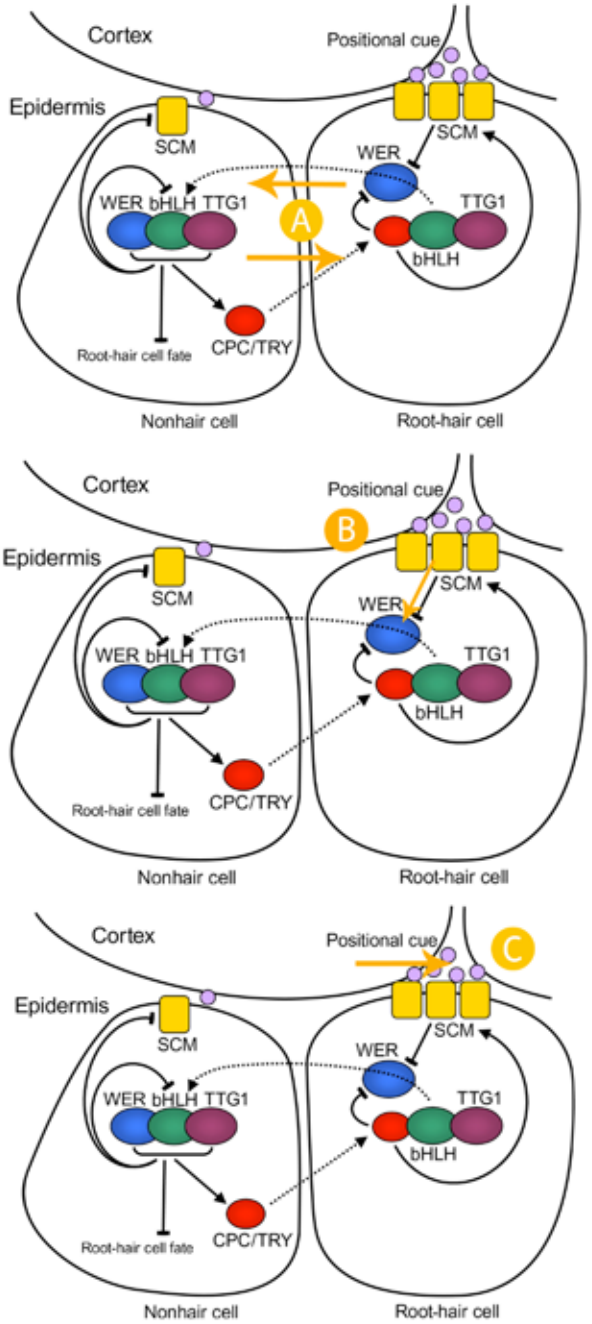


Figure 5. Models of class XI myosin action in cell fate determination. (A) MYA1 may be involved in the trafficking of transcription factors between epidermal cells through the plasmodesmata. (B) MYA1 may be involved in transporting signals between SCM and WER. (C) MYA1 may transport a positional-cue to the apoplast between cortical cells to induce the hair cell fate in neighboring epidermal cells. Diagrams modified after (Kwak and Schiefelbein, 2008).



position is not reduced and therefore can lead to repression of *WER*, which results in future ectopic hair cells. Additionally, MYA1 may be involved in the preferential localization of the positional cue from the cortex to the epidermis (Figure 5C). SCM is only preferentially expressed in hair cells once cell fate has already been determined (Kwak and Schiefelbein, 2008). In other words, preferential localization of the positional cue is critical in initiating cell patterning early in development when SCM accumulates at a similar level in both hair and non-hair cells. Thus, ectopic root hair growth may occur in mutants when the positional cue incorrectly activates SCM in cells in the N-position.

In this study, multiple reporter-gene constructs were developed to identify the approximate location in the signal transduction pathway where *MYA1* may transport a transcriptional regulator. Three promoter-GUS (beta-glucuronidase) constructs — *WER::GUS*, *EGL2::GUS*, and *GL2::GUS* — were used to identify any defects in their spatial arrangement in the patterning stage of root hair development.

Materials and Methods

Plant lines

A single type of *Arabidopsis* T-DNA insertion line of *MYA1* was used: *mya1-5*, which has been shown to be a null-mutant (Park, 2010). The seeds were obtained from Salk Institute Genomic Analysis Laboratory (http://signal.salk.edu). Additionally, the three promoter-GUS constructs were acquired from John Schiefelbein of the University of Michigan and were described in his previous study (Schiefelbein, 2003). Since the T-DNA insertions were developed in the Columbia ecotype (*Col-0*), the three different promoter-GUS lines were previously crossed into *Col-0* (Park, 2010).

Subsequently, each of the three promoter-GUS lines in the *Col-0* ecotype was crossed with *mya1-5*, and the F1 progeny was allowed to self-pollinate (Park, 2010). In the following (F2) generation, the presence of homozygous T-DNA insertions in *mya1* was confirmed by PCR. The primers used were the following:

MYA1-specific:

5' TCCACAAAGTGCTGGATTCCC -3' (forward),

5'-TGTGTACCGTATTTGTCGTCCCA-3' (reverse);

T-DNA-specific:

5'-TCCACAAAGTGCTGGATTCCC-3' (forward),

5'-TGGTTCACGTAGTGGGCCATCG-3' (reverse).

Similarly, reporter expression tests were used to confirm the presence of promoter-GUS fusions.

Seed preparation and seedling growth conditions

Seeds were sterilized in 30% bleach and 0.1% Triton X-100 for 10 minutes and were rinsed four times with autoclaved water. Sterilized seeds were then plated onto square petri dishes with 0.25 x Murashige and Skoog (1/4 MS) basal salt mixture, 0.5% phytagel, and 1% sucrose at pH 5.7-6.0. On each plate, approximately 15 seeds of one reporter-gene construct in the mutant background were plated along with 5 seeds of the same reporter-gene construct in the wild-type background. The seeds were then allowed to germinate and grown vertically for 4-6 days in the growth chamber.

Reporter-gene expression

The histochemical assay of each promoter-GUS construct was performed differently in order to optimize their clarity of expression:

For *WER::GUS* constructs (including both mutant and wild-type backgrounds), seedlings were immersed in GUS staining solution without X-Gluc for 40 minutes. The staining solution consists of the following ingredients in a 10 ml solution: 5 ml of 100 mM sodium phosphate buffer, 200 μl of 10% Triton X-100, 800 μl of 100 mM potassium ferrocyanide, 800 μl of 100 mM potassium ferricyanide, and autoclaved water for the remainder. Subsequently, the same composition and volume of GUS staining solution, except with 400 μl of 100 mg/ml X-Gluc, was added to the previous solution and allowed to incubate with the seedlings for 30 minutes. Following pre-incubation and incubation, each seedling was first placed in 100% ethanol. Then, the ethanol was replaced with water in a five-step dilution series (75%, 50%, 30%, 15%, and 0%). The seedlings were later moved and immersed in 50% glycerol for 1 to 2 hours.

For *EGL3::GUS* constructs, seedlings were incubated in GUS staining solution with X-Gluc for 20 minutes. The staining solution consists of the following ingredients in a 10 ml solution: 5 ml of 100 mM sodium phosphate buffer, 200 μl of 10% Triton X-100, 80 μl of 100 mM potassium ferrocyanide, 80 μl of 100 mM potassium ferricyanide, 100 μl of 100 mg/ml X-Gluc, and autoclaved water for the remainder. Following pre-incubation and incubation, each seedling was placed in 100% ethanol. Then, the ethanol was replaced with water in a five-step dilution series (75%, 50%, 30%, 15%, and 0%). The seedlings were later moved and immersed in 50% glycerol for 1 to 2 hours.

For *GL2::GUS* constructs, seedlings were immersed in GUS staining solution without X-Gluc for 15 minutes. The staining solution consists of the following ingredients in a 10 ml solution: 5 ml of 100 mM sodium phosphate buffer, 200 μl of 10% Triton X-100, 160 μl of 100 mM potassium ferrocyanide, 160 μl of 100 mM potassium ferricyanide, and autoclaved water for the remainder. Subsequently, the same composition and volume of GUS staining solution, except with 400 μl of 100 mg/ml X-Gluc, was added to the previous solution and allowed to incubate with the seedlings for 15 minutes. Following pre-incubation and incubation, each seedling was placed in 100% ethanol. Then, the ethanol was replaced with water in a five-step dilution series (75%, 50%, 30%, 15%, and 0%). The seedlings were later moved and immersed in 50% glycerol for 1 to 2 hours.

Microscopy

In order to assess the expression patterning of each promoter-GUS construct, each seedling was placed on a microscope slide, enclosed with a glass cover, and viewed under a Leica stereomicroscope (Leica MZ16FA, http://www.leica-microsystems.com). Images were captured with a digital camera (Leica DFC420) and viewed with its corresponding software (Leica FW4000).

Statistical analyses

With the aim of determining the rate of ectopic *GL2::GUS* non-expression in wild-type and mutant seedlings, Photoshop CS2 (Adobe Systems, Inc.) was used to mark the number of unstained cells in the N-position, the number of unstained cell files, and the number of cells per file. The numbers for each seedling were then substituted in the following formula:

$$Rate = \frac{Number\ of\ unstained\ cell\ pairs}{Number\ of\ unstained\ cell\ files \times Number\ of\ cells\ per\ file}$$

Approximately 20 seedlings were counted and calculated for each genetic background.

Results

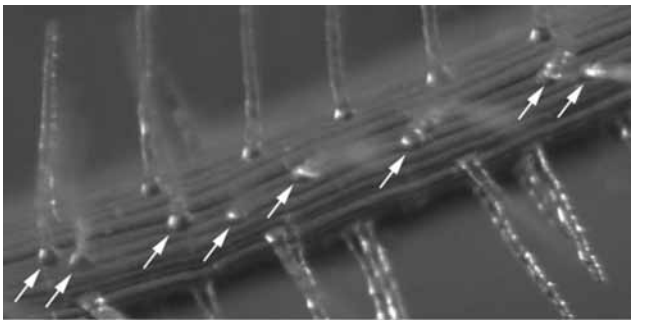
GL2::GUS was first used for expression patterning comparisons

It has been previously demonstrated in *Arabidopsis* that *mya1-5* mutants developed more root hairs than wild-type. More specifically, quantification of root hair density reveals that *mya1-5* developed approximately 20% more root hairs per millimeter than wild-type. This difference has been shown to be statistically significant (t-test, $p<0.005$). Moreover, the increase in root hair density can be attributed to ectopic root hair growth rather than a possible decrease in epidermal cell size. Highly magnified images of *mya1-5* roots exhibit not only normal cell lengths but also a frequent pairing of root hairs in adjacent cells (Figure 6) (Park, 2010). This sort of pairing rarely occurs in wild-type as longitudinal hair cell files are usually sandwiched between two non-hair cell files. These two observations suggest that *MYA1* may have a role in transporting transcriptional regulators that determine epidermal cell fate in *Arabidopsis* (Dolan, 2006).

To determine if any signals are being transported by *MYA1*, reporter-genes were used to see if patterning of gene expression in *mya1-5* mutants was different from those in wild-type. The following three promoter-GUS constructs were introduced into both wild-type and mutant backgrounds: *WER::GUS*, *EGL3::GUS*, and *GL2::GUS*. Three constructs were used to limit the range of possible cargoes *MYA1* may carry as each construct represents a different location in the root hair patterning pathway. In particular, WER function occurs at the beginning of the pathway, while GL2 regulates near the output of the pathway, and EGL3 acts in between the two (Kwaka and Schiefelbein, 2007).

The expression patterning of all three promoter-GUS fusions was determined by staining for GUS activity. Staining conditions had to be optimized in the wild-type background before any comparisons could be made with the mutants. As a result, the GUS concentrations of the staining solutions and incubation durations of the seedlings differed among the reporter gene fusions. In particular, the conditions for *GL2::GUS* expression were the most flexible, while the provisions for *EGL3::GUS* and *WER::GUS* were progressively more confining. After finding the ideal conditions for each, *GL2::GUS* expression appeared to be the most consistent and distinct, and thus, it was chosen to be the first construct used in patterning comparisons. In addition to its advantages in staining quality, *GL2::GUS* was also a convenient choice as GL2 regulates near the output of the pathway

Figure 6. Effects of *MYA1* mutation on root hair growth. This is the proximal section of the root in a 4-day-old seedling taken under visible light. Arrows indicate paired root hairs. Image reproduced from (Park, 2010).



(Guimil and Dunand, 2006). If *MYA1* has any role in regulating epidermal cell fate through this pathway, *GL2::GUS* expression patterning would certainly be different in the mutant background.

GL2::GUS* expression was not affected by *MYA1

The GL2 gene encodes for a homoedomain transcription factor that activates an inhibitory pathway to determine the non-hair cell fate. Thus, *GL2::GUS* expression normally occurs in epidermal cells located in the N-position (Lee and Schiefelbein, 2002). Since *mya1-5* mutants frequently grow ectopic root hairs, one predicts to see *GL2::GUS* expression frequently inhibited in cells of the N-position in *mya1-5*.

To test this hypothesis, several seeds from each background — *GL2::GUS* and *mya1-5* x *GL2::GL2* — were allowed to germinate and to grow on vertical plants containing a defined growth medium. Four- to six-day-old seedlings were then histochemically assayed in GUS staining solution and viewed under a stereomicroscope. Epidermal cells on the root tips were later examined for *GL2::GUS* expression. This region of the root is where the establishment of cell patterning occurs, which is also the region of interest. The morphogenesis phase (i.e. the growth of tubular appendages), on the other hand, occurs towards the proximal section of the root. After examination, no obvious differences in *GL2::GUS* expression patterning between wild-type and *mya1* mutants were found (Figure 7A). Both backgrounds exhibited normal *GL2::GUS* expression in some roots and slightly distorted staining in others (Figure 7B). However, because some roots in each line showed examples of *GL2::GUS* non-expression in the N-position, it is possible that this ectopic cell type appeared more often in the mutants.

A rate equation was developed to quantify any differences between mutant and wild-type seedlings. The formula calculated the percentage of trichoblasts in the H-position that was paired with a trichoblast in the N-position (Figure 8). To put it simply, the rate represented the number of mistakes in patterning divided by the total number of normal trichoblast cells. This rate was approximately 0.0563 for wild-type and about 0.0554 for *mya1* (Figure 9). The statistical analyses suggested that there was no difference in the rate of ectopic *GL2::GUS* non-expression between the two genetic backgrounds. This finding also suggested that *MYA1* does not transport any of the transcription factors or their upstream regulators found in the pathway.

Figure 7. *GL2::GUS* expression patterns as makers of cell patterning. (A) *GL2::GUS* expression was restricted to specific files of epidermal cells in the N-position for both wild-type and mutant lines. (B) In both genotypes, some roots also had unstained cells in the non-hair cell position, as indicated with red arrows.

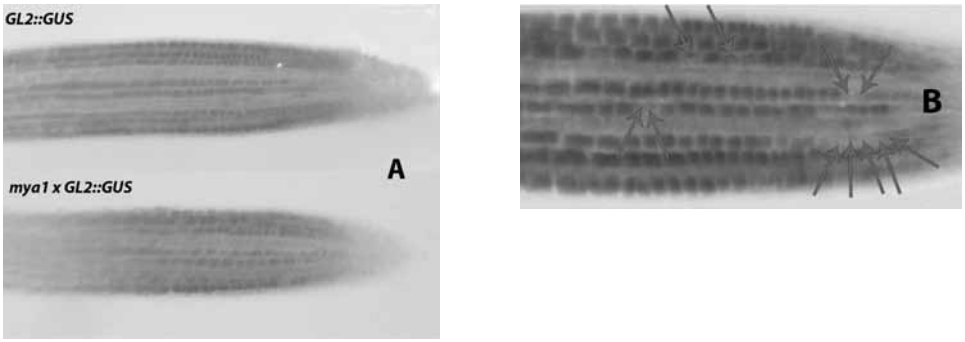
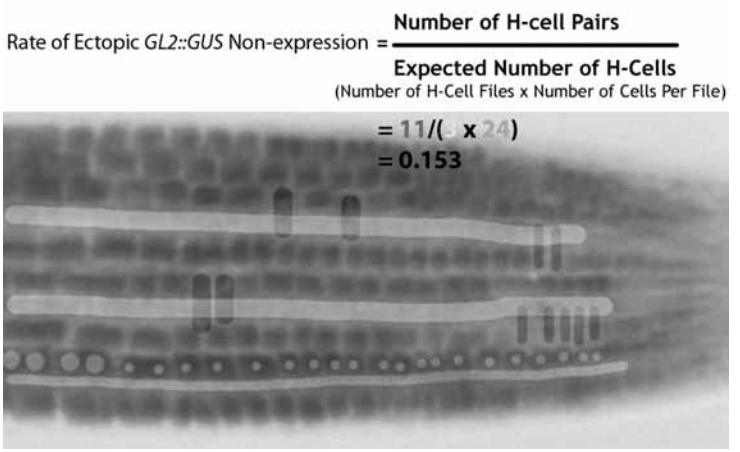
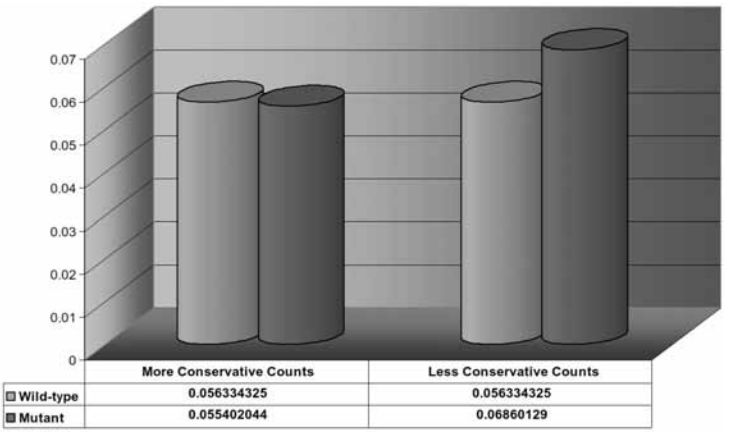


Figure 8. Sample count and calculation of *GL2::GUS* non-expression. The above formula was used to normalize the number of mistakes in patterning in order to account for differences in root size and field of view. As for the stained seedling root, the red overlays represent H cell pairs, while the yellow lines and blue circles signify cell files and cells, respectively.



However, this inference needs to be received with some caution as the counting process was difficult. There were cells that expressed *GL2::GUS* faintly or staining that diffused across the plasmodesmata into neighboring epidermal cells. Furthermore, accurate counting was also predicated on defining cell files — whether they were hair cell files or non-hair cell files. Occasionally, two cell files merged into one cell file as one moved away from the root tip, while other times, one cell file divided into two. This branching of cell files made it difficult to determine whether cells ectopically non-expressed or expressed *GL2::GUS*. Thus, depending on one's definition of a cell file, there may be several pairs of trichoblasts in a row or no pairs at all.

Figure 9. Mean rates of ectopic *GL2::GUS* non-expression. The mean rate between wild-type and mutant seedling roots was approximately the same in the more conservative count. However, the less conservative count suggested a ≈22% increase in ectopic non-expression for mutant seedling roots. This difference was not statistically significant.



Because of all these factors, another data set was recorded in addition to the first count that has already been presented above. In the second count, any borderline decision swayed towards a cell being counted as an ectopic trichoblast. The second data set showed an approximate 22% increase in ectopic *GL2::GUS* non-expression in *mya1* mutants. The second count could potentially be interesting as it paralleled the approximate 20% increase in root hair density found earlier in the mutants (Park, 2010), but the difference was not found to be statistically significant.

Overall, the more conservative count could be analyzed with greater confidence, and thus, there was no difference in *GL2::GUS* expression patterning between wild-type and mutant roots as found in the first and more conservative count regardless of what the second and less conservative count inferred.

Discussion

Prior studies in organelle motility and epidermal cell fate determination suggested the possibility of *MYA1* involvement in the intercellular transport of mobile transcription factors and their upstream signals. Class XI myosins, for example, have been shown to be involved in the trafficking of mitochondria, Golgi stacks, chloroplasts, vesicles, and peroxisomes. These motor proteins may also have a function in the transport of other intracellular structures, such as regulatory proteins and signal molecules (Prokhnevsky et al., 2008). Additionally, specific antibodies of class VIII myosins demonstrate abundant localization to the plasmodesmata (Reichelt et al., 1999). Class XI myosins, thus, may also have a function in regulating transport between plant cells. Studies in epidermal transcriptional regulation also demonstrate the existence of lateral inhibition — a method of intercellular communication where cells differentiating into a specific cell type prevent their neighbors from developing into the same fate. This mechanism of inhibition requires the movement of regulatory signals between epidermal cells (Lee and Schiefelbein, 2002). For instance, both the GL3/EGL3 complex and CPC transcription factor have been shown to move from their cell type of preferential expression to the other cell type in order to exert their regulatory effects (Ishida et al., 2008). With these previous results in mind, it is reasonable to believe that *GL2::GUS* expression patterning would be disrupted in *mya1* given that any displaced signal molecule regulating upstream of GL2 would result in an increased frequency of *GL2::GUS* non-expression in N cells.

However, this study suggested another function for *MYA1*. The data demonstrated that *MYA1* was not required for intercellular movement of any of the transcriptional regulators or signal molecules upstream from GL2, even though *MYA1* mutation disrupted cell-fate determination in the root epidermis. *MYA1*, instead, may act on hormonal or environmental signals that could override the default cell fate determined by transcriptional regulation. The former presents a possible role for myosin proteins given the important presence of hormones during root hair development. More specifically, *MYA1* may be involved in the movement of or responses to auxin or ethylene, both of which are positive regulators of root hair growth (Schiefelbein et al., 1997). Similarly, the transport of environmental signals can be affected by the presence of *MYA1*. Nutrient stresses, such as phosphate and iron deficiencies, have been known to result in longer and ectopic root hairs (Guimil and Dunand, 2006). According to this notion, *mya1-5* mutants may induce partial nutritional starvation by reducing the roots' ability to carry phosphate and iron to their destinations. Overall, *MYA1* may be the link between the default transcriptional pathway and its downstream pathways — hormonal and environmental responses.

Conclusions

This study utilized reporter-gene experimental approaches to test *MYA1*'s possible involvement in the transport of transcriptional regulators and their upstream signal molecules. The data suggested that there was no difference in *GL2::GUS* expression patterning between wild-type and mutant backgrounds, and as a result, *MYA1* must affect epidermal cell fate in a manner separate from the transcriptional regulatory pathway. Future research should analyze the differences in root hair density between *mya1* and wild-type following exposure to various degrees of hormonal (i.e. auxin and ethylene) or nutritional (i.e. phosphate and iron) stresses.

References

Dolan, L. (2006). Positional information and mobile transcriptional regulators determine cell pattern in the Arabidopsis root epidermis Journal of Experimental Botany 57.1, 51-54.

Guimil, S., and Dunand, C. (2006). Patterning of Arabidopsis epidermal cells: epigenetic factors regulate the complex epidermal cell fate pathway. Trends in Plant Science 11.12, 601-609.

Ishida, T., Kurata, T., Okada, K., and Wada, T. (2008). A genetic regulatory network in the development of trichomes and root hairs. Annual Review of Plant Biology 59, 365-386.

Kwak, S.-H., and Schiefelbein, J. (2008). A Feedback Mechanism Controlling SCRAMBLED Receptor Accumulation and Cell-Type Pattern in Arabidopsis. Current Biology 18, 1949-1954.

Kwak, S.-H., Shen, R., and Schiefelbein, J. (2005). Positional Signaling Mediated by a Receptor-like Kinase in Arabidopsis. Science 307, 1111-1113.

Kwak, S.-H., and Schiefelbein, J. (2007). The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis. Developmental Biology 302, 118-131.

Lee, M.M., and Schiefelbein, J. (2002). Cell Pattern in the Arabidopsis Root Epidermis Determined by Lateral Inhibition with Feedback. Plant Cell 14, 611-618.

Li, J.-F., and Nebenführ, A. (2007). Organelle targeting of myosin XI is mediated by two globular tail subdomains with separate cargo binding sites. The Journal of Biological Chemistry 282.28, 20593-20602.

Ojangu, E.-L., Järve, K., Paves, H., and Truve, E. (2007). Arabidopsis thaliana myosin XIK is involved in root hair as well as trichome morphogenesis on stems and leaves Protoplasma 230, 193-202.

Park, E. (2010). Reverse genetic and cell biological approaches to the study of developmental functions of Class XI myosin in *Arabidopsis thaliana*. In Botany (Knoxville: University of Tennesse, Knoxville), pp. 161.

Prokhnevsky, A.I., Peremyslov, V.V., and Dolja, V.V. (2008). Overlapping functions of the four class XI myosins in Arabidopsis growth, root hair elongation, and organelle motility. PNAS 105.50, 19744-19749.

Reichelt, S., Knight, A.E., Hodge, T.P., Baluska, F., Samaj, J., Volkmann, D., and Kendrick-Jones, J. (1999). Characterization of the unconventional myosin VIII in plant cells and its localization at the post-cytokinetic cell wall The Plant Journal 19.5, 555-567.

Schiefelbein, J. (2003). Cell-fate specification in the epidermis: a common patterning mechanism in the root and shoot Cell-fate specification in the epidermis: a common patterning mechanism in the root and shoot **6,** 74-78.

Schiefelbein, J.W., Masucci, J.D., and Want, H. (1997). Building a Root: The Control of Patterning and Morphogenesis during Root Development. The Plant Cell 9, 1089-1098.

About the Author

Kevin Kuo received his B.S. in the biological sciences, concentrating in Biochemistry and Cellular and Molecular Biology, from the University of Tennessee in 2010. He is currently a first-year dental student at the University of Michigan School of Dentistry. Kevin's professional interests include geriatrics, endodontics, and biochemical research.

About the Advisor

Dr. Andreas Nebenführ is an associate professor in the Department of Biochemistry and Cellular and Molecular Biology. His research interests are centered around transport processes inside plant cells with a particular focus on the mechanisms responsible for rapid cytoplasmic streaming. These movements of cellular components are driven by myosin motor proteins, nano-scale machines that are also responsible for muscle contraction in humans. Research in the lab involves a variety of different approaches from genetics over molecular biology and biochemistry to cell biology and plant physiology. Dr. Nebenführ benefits from a large number of undergraduate students who have helped with this research over the years. He also enjoys teaching the General Genetics class in which he introduces biology majors to the sometimes quite abstract world of information processing in living organisms.