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

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

By

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In Partial Fulfillment of the Requirements for the Degree of Bachelor of Science
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.
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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 in 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 is possible due to the roots’
transparency and ability to grow on defined media. Even more important to these researchers is the fact that 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 above 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 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

**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).
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, resulting 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, class XI isoforms demonstrate numerous structural similarities with

![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.](image-url)
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 (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 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.

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).
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:

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

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

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*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).
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 (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

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**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.
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 8. Sample count and calculation of GL2::GUS non-expression.](image)
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 $\approx 22\%$ increase in ectopic non-expression for mutant seedling roots. This difference was not statistically significant. 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.

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


