

Root hair patterns and gene expressions on myosin XI and werewolf, glabra 2 and enhancer of glabra 3 mutants

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Abstract. In the model plant *Arabidopsis thaliana* (mouse-ear cress), the root hair cells of the root epidermis are arranged in a longitudinal striped pattern. Some of these epidermis cells are hair cells and some develop as non-hair-bearing epidermal cells. Adjacent epidermal cells are continuously communicating with each other through regulatory genes and determine which epidermis cells express root hairs and which do not. This communication between the adjacent epidermis cells gives the cells this regular root hair pattern. Myosin act as motors in the cell and move the organelles and protein complexes inside of the cell.

Myosin mutants have more root hairs and it is because they form extra root hairs on the non hair cells epidermis. The *WER* (*werewolf*), *GL2* (*glabra 2*), and *EGL3* (*enhancer of glabra 3*) genes control cell patterning in the *arabidopsis* root epidermis. In the lab expression patterns of these genes were observed in root tips of myosin mutants and wild type.

With a combination of genetic and morphological approaches, my research addressed two questions of how two myosin mutants affect the specific patterning of the root hairs and also how they affect the gene expressions of regulatory genes *WER*, *GL2*, and *EGL3*. In a broad perspective my research helped with the understanding of how intracellular transport in a cell affects the intercellular communication in *Arabidopsis*. And the staining of the reporter gene in this experiment revealed the cells that expressed these regulatory genes

Background

In the model plant *Arabidopsis thaliana* (mouse-ear cress), the cells of the root epidermis are arranged in a longitudinal striped pattern. Some of these epidermis cells are hair cells and some develop as non-hair-bearing epidermal cells. Positional signals then initiate cell-specific expression of a number of transcriptional factors. These transcriptional factors complete the patterning process. This results in the expression of the hair promoting genes in hair cells or the repression of the hair gene in the non-hair cells. (1) In young roots, the root epidermis has a ring of 16 initials, which are located to the outside of the columella initials. This ring then makes way for 16 epidermal cell files. Eight of these cell files are in between the uncton of underlying cortical cells. The other

eight cells are directly over a single cortical cell as it can be seen in transverse orientation in Figure 1. The cells over the cortical junctions express root hairs while the cells located over the single cortical cells do not express the hair gene and develop as non-hair-bearing epidermal cells. (1) Normally, hair and non-hair cells follow a strict alternating pattern, which can also be detected by examining the expression of regulatory genes.

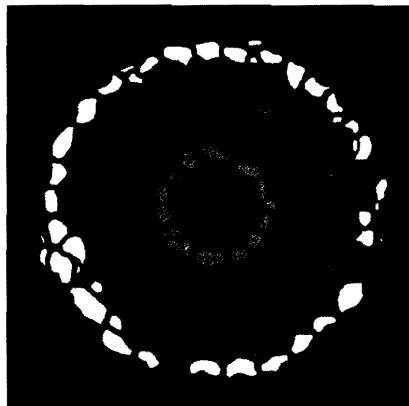


Figure 1

Myosins are actin-based motors known to function in many form of Eukaryotic motility such as cell migration, cytokinesis, phagocytosis, maintenance of cell shape, and organelle trafficking. (2) Myosins in non-plant organisms have been extensively characterized; however, less is known about the presence and functions of myosins in plant cells. However, one of the likely functions of myosins is movement along actin filaments. (3) Myosins move along actin filaments. They function of the myosins in plant cells are related to various processes such as cell division, movement of mitochondria and chloroplasts, cytoplasmic streaming, rearrangement of transvacuolar strands, and statolith positioning. Class XI myosins are represented in *Arabidopsis thaliana* 13 genes. (4) *Arabidopsis* encodes about 13 class XI myosins. The actin cytoskeleton is involved in many processes such as signaling, cell division, organelle trafficking and

morphogenesis in plants. The differentiating root-hair cells, trichoblasts, and the differentiating hairless cells, atrichoblasts, can be distinguished in different ways such as their cytoplasmic density, vacuole formation and extent of elongation. (5) The differentiation activity in the root cells happen early but a change in the immature epidermal cells can also induce a change in its developmental fate. The cell position and cell type differentiation have a correlation to each other. This correlation implies that the cell-to-cell communications are critical for establishing the appropriate cell patterns. (6)

In Arabidopsis, several genes have been used in establishing the root epidermal pattern. Genes such as *GLABRA2 (GL2)*, *WEREWOLF(WER)*, and *CAPRICE(CPC)*. The *GL2* gene encodes a homeo-domain transcription factor protein. This transcription protein is required to specify the non-hair cell type. Accordingly, the *GL2* gene is expressed preferentially in the non hair cell position during epidermis development. The *WER* gene encodes a MYB transcription factor. This transcription factor is required for specifying the non hair cell type. This *WER* expression occurs mostly in the developing epidermal cells in the non-hair position of the root. *WER* is also a positive regulator of *GL2* expression. The *CPC* gene encodes a small MYB protein without a putative transcriptional activation domain that is required for hair cell specification. But more recent findings have shown that *CPC* is mostly expressed in the non hair cell position. (6)

Mutations in the *GL2* gene have been found to specifically alter the differentiation of the hairless epidermal cells. This causes the hairless cells to produce root hairs, which indicates that *GL2* affects epidermal cell identity. Detailed analyses of these

differentiating cells showed that, despite forming root hairs, they are similar to atrichoblasts of the wild type in their cytoplasmic characteristics, timing of vacuolation, and extent of cell elongation. (5) Experiments have showed that the GL2 gene also prefers be expressed in the differentiating hairless cells of the wild type, during a period in which epidermal cell identity is believed to be established. These results indicate that the GL2 homeodomain protein normally regulates a subset of the processes that occur during the differentiation of hairless epidermal cells of the *Arabidopsis* root. More specifically, GL2 acts in a cell-position-dependent manner to suppress hair formation in differentiating hairless cells. (5)

As seen in the cross section of the root in Figure 2 (7), the epidermis cells that express the EGL3 (enhancer glabra 3) gene and will become root hairs are stained. In Figure 3 (8) one can see the stained cells occur in a regular pattern. Adjacent plant cells are continuously communicating with each other through regulatory genes and determine which epidermis cells express root hairs and which do not. This communication between the adjacent epidermis cells gives the cells this regular root hair pattern. In previous research myosin mutant roots were shown to have root hairs on adjacent epidermis cells.

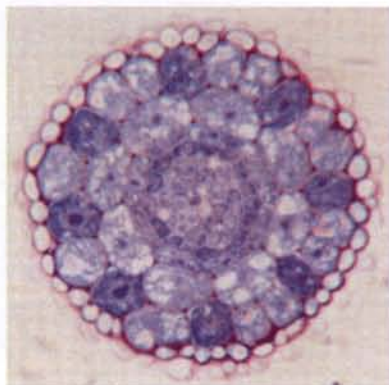


Figure 2

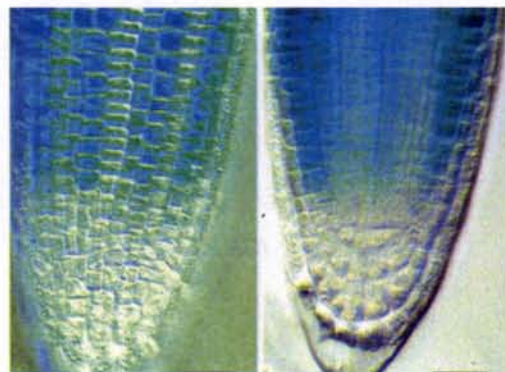


Figure 3

Many of these putative transcriptional regulators are known to influence the cell fate decision. Studies have shown that with the maize *R* gene has been used to suggest that a bHLH transcription factor also participates in this process. The *Arabidopsis* genes encoding bHLH proteins, *ENHANCER OF GLABRA3* (*EGL3*), acts in a partially redundant manner to specify root epidermal cell fates. Plants homozygous for mutations in this and few other genes fail to specify the non-hair cell type, whereas plants overexpress either gene produce ectopic non-hair cells. We also see that these genes are required for appropriate transcription of the non-hair specification gene *GL2* and the hair cell specification gene *CPC*, showing that the *EGL3* gene influences epidermal cell fates.(2)

The *WER* gene encodes a MYB-type protein and is preferentially expressed within cells that are destined to adopt the non-hair fate. Furthermore, *WER* is shown to regulate the position-dependent expression of the *GLABRA2* homeobox gene, to interact with a bHLH protein, and to act in opposition to the *CAPRICE* MYB. (6)

Experimental Procedures

Crossing WER::GUS, GL2::GUS, and EGL3::GUS lines with the myosin XI mya 1 allele

The *WER::GUS, GL2::GUS, and EGL3::GUS* line seeds were obtained. These seeds along with *Columbia* wild type and the myosin XI *mya-1* gene seeds were sterilized, and plated. The roots were allowed to grow on the MS agar medium plates for about five to seven days in a day and night cycle lighted growth chambers. After each root was about one to two inches all the lines were transformed. At least 5 lines of each allele were planted and allowed two months to grow. After the plants each just flower,

the promoter-reporter constructs for the *WER::GUS*, *GL2::GUS*, and *EGL3::GUS* regulatory genes were introduced to myosin mutant lines and crossed with *mya 1* lines. The seeds from these crosses were then harvested and then *GUS* stained and planted. These plants were left to self-fertilize and to obtain F2 generation plants from which homozygous lines are to be selected and isolated.

Whole-Mount Gus Staining to Stain for Reporter Gene (GUS) activity

Once the crosses were obtained the *GUS* activity of the genes was determined using the Gus staining method. The seeds were sterilized and plated in MS agar media. After growing about one to two inches each root tissue was stained as follows. The tissues were placed in cold 90% Acetone until all samples were harvested. The roots were then incubated for 20 minutes each at 4°C. While the tissues incubated a staining buffer is freshly prepared using pre-chilled solutions and stored on ice.

Staining buffer (50 ml)	
0.1 M sodium phosphate buffer (pH 7.2)	25 ml
5% Triton X-100	2.5 ml
500 mM potassium ferrocyanide	50 µl
500 mM potassium ferricyanide	50 µl
dH ₂ O	22.4 ml

The samples are then washed in staining buffer at least three times. The staining solution is then prepared by adding previously prepared X-Gluc stock solution.

X-Gluc Stock Solution (store in dark at -20°C)

100mM in N,N-dimethylformamide (DMF)
Use 500 µl in 50 ml of Staining Buffer

The staining buffer is then removed and the staining solution is added. The *WERxmya 1* and *EGL3xmya 1* crossed roots were incubated for 30 minutes at 37°C while the *GL2xmya 1* line was incubated for 15 minutes at the same temperature.

The staining buffer is then removed and the samples are each washed twice with 70% ethanol at room temperature, each round allowing the sample to sit for 10 minutes. The samples are then washed with 95% ethanol at room temperature for 30 minutes. All the ethanol is then removed and fresh 95% ethanol is added to the samples in which they can remain in until further research is being done.

The samples are then examined and selected for mutant phenotype. The homozygous lines are picked out by selecting the lines in which all the sampled roots are stained. Using a microscope with an attached camera the stained roots for the homozygous lines were more closely looked at the pictures were taken from the root tips of these tissues. Pictures were also taken from the root tips of the individual *GUS* lines, wildtype, *mya 1*, and the crossed *WERxcol*, *GL2xcol*, and *EGL3xcol* lines as well for control and to compare the roots to find out whether the expression of the regulatory genes followed different patterns.

Polymerase Chain Reaction (PCR) and Gel Extraction method to select homozygous genotypes of mya1 crosses

Once the crosses are made and the homozygous lines are picked through selection based on the staining of the roots, the homozygous lines are genotyped using PCR and gel extraction methods. In the PCR method the extracted cDNA of the plant tissue is used and the PCR reaction is prepared as shown in the table.

PCR Reaction Mixture

Component	Volume
Hifi Buffer	2.5 μ L
DNA template	1.0 μ L
Oligonucleotide Forward Primer	0.5 μ L
Oligonucleotide Reverse Primer	0.5 μ L
dNTP mix	0.5 μ L
Taq Polymerase enzyme	0.2 μ L
ddH ₂ O	19.8 μ L

The Gel extraction method is performed using 1% agarose gel ran for 30 minutes on 100V to obtain the bands.

Results and Data

Root Hair Tips of the Control and Mutated Arabidopsis Genes.



Figure 3

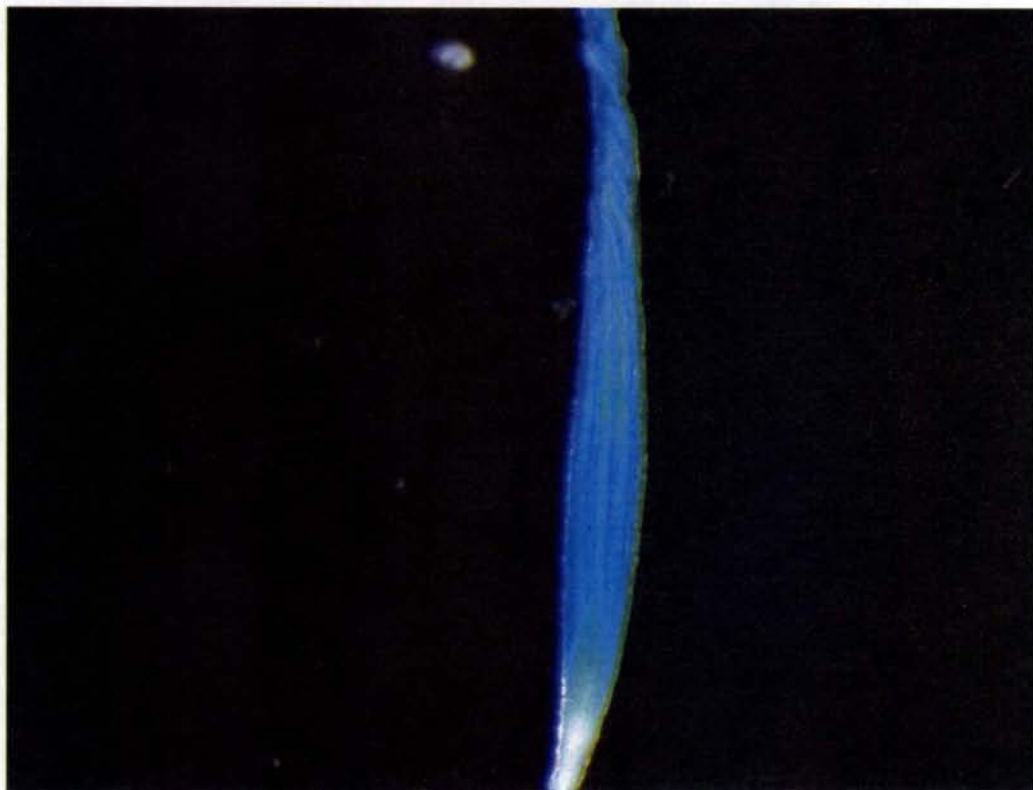


Figure 5



Figure 6

In Figure 4 the *colxGL2* cross can be seen and the darker streaks of hair cells can be seen in a regular pattern with no hair cells appearing next to each other as excepted for the control gene.

In Figure 5 and 6 the *WERxmya 1* and *EGL3xmya 1* crosses are seen respectively. In the two pictures it can be seen that the cells that are signaling for and showing hairs are darker. On both graphs it can be seen that there are two darker cells, ones signaling for hair, located right next to each other with one or two non-hair cells separating another set of hair signaling cells next to each other.

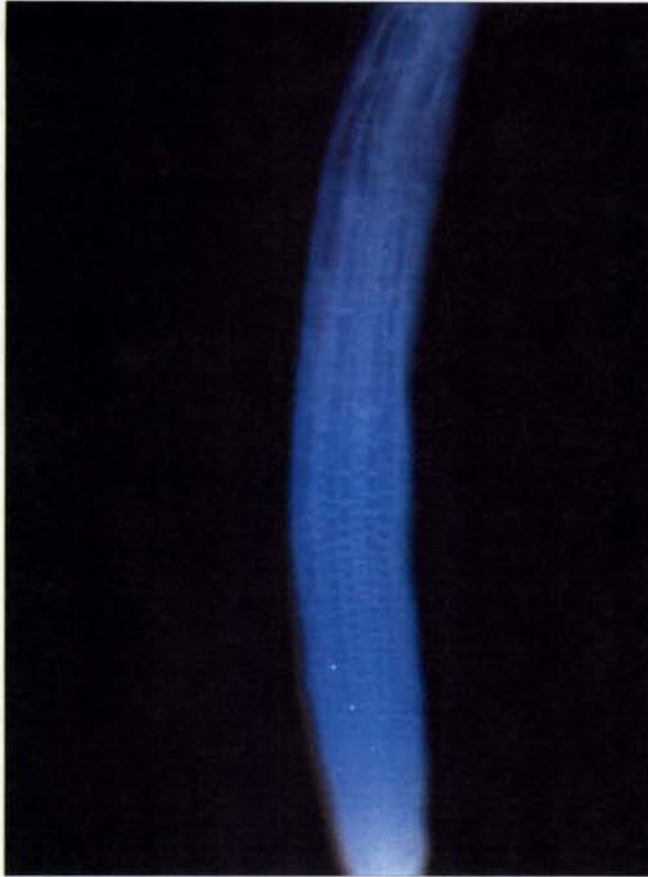


Figure 7

In figure 7 the *GL2xmya 1* cross's cell can be seen. It can be clearly seen that there are two darker stained cells, which are hair-signaling cells. The two adjacent cells both signal for hair unlike the control and wild type who have at least one or two non hair signaling cells in between every hair cell.

Figure 8

In figure 8 another picture of the *GL2xmya 1* gene cross can be seen. This picture also clearly shows the two hair signaling cells adjacent to each other and shows a different phenotype from the wild type and control cells.



Future Experiments

The experiments and results showed that the *WERxmya 1*, *GL2xmya 1* and *EGL3xmya 1* gene crosses between the regulatory genes with the GUS lines and the myosin XI showed a perturbed pattern in their root's cell hair growth. The mutants all showed adjacent hair cells growing. This pattern did not follow the wild-type hair growth pattern, which never signals for hair cells to grow adjacent to each other.

Next in this experiment the root hair patterning genes on these myosin mutants need to be further investigated. These mutant genes need to be genotyped and using the cross sectioning of these roots the hair and non hair cell patterns can be further investigated to find the specific affect of the myosin motor and its effect on the ectopic cells and the patterns it creates to make certain cells express or not express the hair gene.

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