

Plant Growth and Reproduction

The Control of Patterning in Plant Root Development

Did you ever think of how a root grows? Down in the dark, with gravity its only cue, the very tip of the root elongates, periodically forming a node from which root branches will extend. How does the root determine the position of its branches, and the spacing between them? The serial organization of the root's branches is controlled by events that happen on a microscopic scale out at the very tip of the root, the so-called root apex. There, within a space of a millimeter or less, molecular events occur that orchestrate how the root will grow and what it will be like.

The problem of understanding how a plant's root apex controls the way a root develops is one example of a much larger issue, perhaps the most challenging research problem in modern botany: What mechanism mediates central pattern formation in the plant kingdom? Almost nothing was known of these mechanisms a decade ago, but intensive research is now rapidly painting in the blank canvas.

Much of the most exciting research on plant pattern formation is being performed on a small weedy relative of the mustard plant, the wall cress *Arabidopsis thaliana* (see photo above). With individual plants no taller than your thumb that grow quickly in laboratory test-tubes, *Arabidopsis* is an ideal model for studying plant development. Its genome, about the size of the fruit fly *Drosophila*, has been completely sequenced, greatly aiding research into the molecular events underlying pattern formation.

To gain some insight into the sort of research being done, we will focus on work being done by John Schiefelbein and colleagues at the University of Michigan. Schiefelbein has focused on one sharply defined aspect of plant root pattern formation in *Arabidopsis*, the formation of root hairs on the epidermis, the outer most layer of cells. These root hairs constitute the principal absorbing surface of the root, and their position is under tight central control.

In a nutshell, the problem of properly positioning root hairs is one of balancing cell production and cell differentiation. Cells in the growth zone beneath the surface of the root—a sheath called a meristem—are constantly dividing. The cells that are produced by the meristem go on



Arabidopsis thaliana. An important plant for studying root development because it offers a simple pattern of cellular organization in the root.

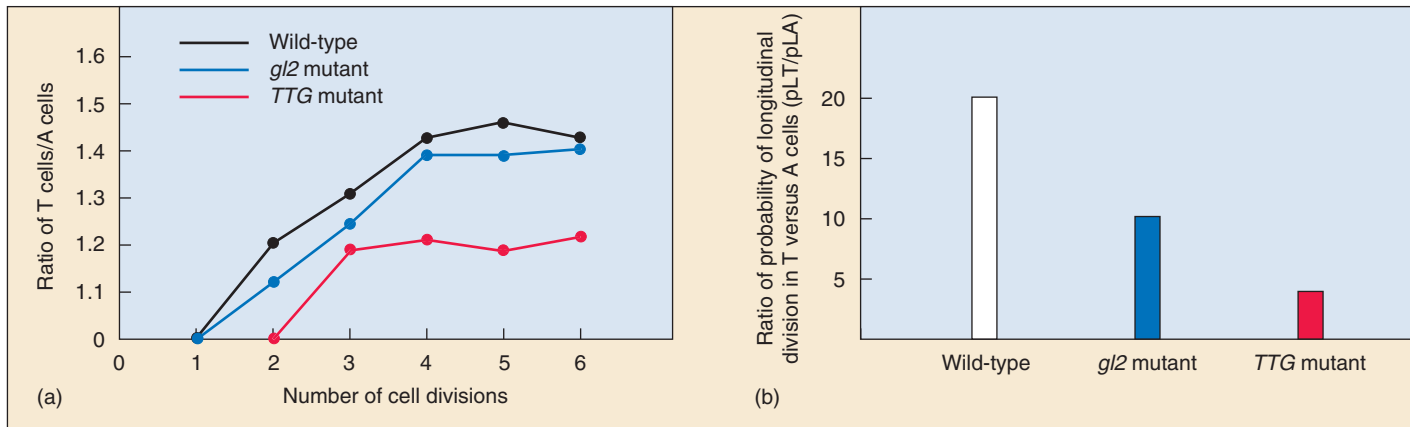
to differentiate into two kinds of cells: trichoblasts which form hair-bearing epidermal cells, and atrichoblasts which form hairless epidermal cells. The positioning of trichoblasts among atrichoblasts determines the pattern of root hairs on the developing root.

When researchers looked very carefully at the dividing root meristem, they found that the initial cells that produce trichoblasts and atrichoblasts alternate with one another in a ring of 16 cells around the circumference of the root. As the cells divide, more and more cells are added, forming columns of cells extending out in 16 files. As the files extend farther and farther out, occasional side-ways divisions fill in the gaps that develop, forming new files.

Maintaining this simple architecture requires that the root maintains a tight control over the plane and rate of cell division. Because this rate is different for the two cell types, the root must also control the rate at which the cell types differentiate. Schiefelbein set out to learn how the root apex coordinates these two processes.

To get a handle on the process, Schiefelbein seized on a recently characterized root pattern mutant called *transparent testa glabra (TTG)*. This mutant changes the pattern of root hairs in *Arabidopsis*, and it has been proposed that it controls whether a cell becomes a trichoblast or an atrichoblast. But does it control the rate and orientation of cell division in the root meristem epidermis?

To answer this question, Schiefelbein's team used clonal analysis to microscopically identify individual cell types in the root epidermis, and set out to see if they indeed divide at different rates, and if the *TTG* mutation affects these rates differently. If so, there must be a link between cell differentiation and the control of cell division in plants.



Comparing the differentiation and cell division of trichoblast (T) cells versus atrichoblast (A) cells in root epidermis. (a) As cell divisions proceeded, T cells and A cells were identified in the root epidermis of wild-type plants and two mutants, *gl2* and *TTG*. Comparing the ratio of T cells to A cells, there is an increase in the number of A cells compared to T cells in the *TTG* mutant. (b) The rate of cell division was also examined by comparing the ratio of probabilities of longitudinal anticlinal cell division in T cells and A cells among the wild-type and mutant plants. This ratio was lowest in *TTG* mutants, indicating that this mutation affects cell division.

The Experiment

Two developmental mutants of *A. thaliana* were used to investigate whether the control of cell differentiation and the rate of cell division were linked. One, *TTG*, alters early events in root epidermal cell differentiation, while the other, *glabra2* (*gl2*) acts later.

The investigators first set out to map the surface of the roots of each mutant type, as well as those of a nonmutant wild type. To avoid confusion in studying files of cells, it is necessary to clearly identify the starting point of each file of cells. To do this, roots were selected that contained clones of trichoblast and atrichoblast produced by longitudinal cell divisions perpendicular to the surface of the root. Called longitudinal anticlinal cell divisions, these clones are rare but easily recognized when stained with propidium iodide. Careful mapping of individual cells with a confocal microscope allowed investigators to determine the number and location of trichoblast and atrichoblast cells present in the epidermal tissue of each clone.

The Results

The researchers made two important observations based on their visual identification of individual trichoblast and atrichoblast cells in the various plant types examined.

1. *The two cell types are produced at different rates.* Among plants that had been cultured for up to six cell divisions, researchers observed a significant difference in the ratio of trichoblast (T) versus atrichoblast (A) cells following two or more cell divisions. You can readily see that the *TTG* mutant produces a significantly lower ratio of T cells to A cells compared to the wild-type plants or *gl2* mutants (see graph *a* above). This strongly suggests that *TTG* is involved in controlling the rate of cell division in the T cell file.

2. *TTG controls the rate of longitudinal cell division.* The research team went on to examine longitudinal cell divisions

that fill in the gaps as cell division causes files of cells to extend outward from the meristem. The researchers set out to determine the probability of such longitudinal anticlinal cell division occurring in the three types of plants shown in graph *a*. The more rapidly cell files are produced, the more often longitudinal divisions would be required to fill in gaps between files. For proper root hair position to be maintained, the rate of this longitudinal division would have to be tightly coordinated with the rate of vertical division within the file.

The investigators found that longitudinal cell division, always rare, but when it did occur, was usually seen, in T cell files. Did the *TTG* mutation affect this process as well as file-extending cell divisions? This was determined by examining the ratio of the probability of longitudinal anticlinal divisions in T cells versus A cells (pLT/pLA).

Researchers compared the ratio in wild-type plants with that in the two mutants, *TTG* and *gl2*. Did the *TTG* mutation alter longitudinal division? Yes! Their results indicate at least a 60% reduction in the pLT/pLA ratio of the *TTG* mutant compared to wild type and *gl2* plants (see graph *b* above). The A file clones of the *TTG* mutants were twice as likely to exhibit this type of cell division than that seen in the wild-type or *gl2* mutants.

This observation directly supports the hypothesis that the *TTG* gene is not only required for cell division in the T cell file, but also controls longitudinal cell divisions which are characteristically more frequent in trichoblasts.

The research team concluded from these studies that *TTG* is probably the earliest point of control of root epidermis cell fate specification, and that this control most likely acts by negatively controlling trichoblast cell fate.