Researchers identify new factors that guide organization of plant roots

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As the root of a growing plant pushes its way through soil, its cells have a lot of organizing to do. New cells must take on the appropriate identities and positions to form distinct layers of tissue that give the root its structure, protect it from the environment, and ensure that it can properly transport materials to and from the rest of the plant.

Howard Hughes Medical Institute scientists have now identified a set of proteins that plays a surprisingly broad role in guiding this tissue formation. The factors, known as the BIRDs, help a root maintain its organization as it grows, guiding several distinct steps in the development of two interior layers of tissue. The study was led by Philip Benfey, an HHMI-Gordon and Betty Moore Foundation investigator at Duke University. Benfey and his colleagues published their findings on October 23, 2015, in the journal *Science*.

Much developmental biology research has focused on figuring out how body plans are established during embryonic development. In animals, the body plan sets up the basic form of the mature adult. But the embryos that form inside plant seeds bear little resemblance to the organisms they will become. "In plants, a lot of what we consider developmental biology happens post-embryonically," Benfey says. That means that plants must continue to specify the identity of new cells and maintain tissue patterns after embryonic development is complete.

Benfey wants to know what controls those patterning processes. His research has focused on how cells in a growing plant become the specialized cell types that comprise a root's four concentric cylinders of tissue. Each layer of tissue in the root is specialized for distinct functions, from the outermost epidermis, which serves as the root's interface with its environment, to the innermost vascular cylinder, which transports water and nutrients.

Between the epidermis and the vascular cylinder are the cortex and the endodermis. The endodermis generates a waterproofing structure called the Casparian strip, whereas the cortex provides structural support for the root (and likely carries out specialized functions of its own). Both layers, which together are known as ground tissue, are formed from stem cells clustered in the very tip of the root.

Unlike cells in a developing animal, plant cells don't move in relation to one another—and that has given developmental biologists something of a headstart in determining exactly how a root forms. Once a cell is born, it is stuck in the same position, and so the cells essentially line up as a developmental timeline, Benfey explains: the youngest cells stay at the tip of a root, and cells next to and above the original cell are progressively more mature.

That's helped scientists figure out the following sequence of events: To create new tissue for an elongating root, a stem cell divides, replenishing the root tip's stem cell niche while also generating a daughter cell above it. That daughter cell divides along the longitudinal axis of the root, so that the two resulting cells are positioned side-by-side. The inner cell of the pair becomes the first cell of the root's endodermis, and the other cell forms the beginnings of the cortex. Those cells then amplify to form the two layers of ground tissue.

Benfey and his colleagues have learned that two factors they call SHORT-ROOT and SCARECROW work together to initiate this process, directing a stem cell to divide into two non-identical cells.

They've found other factors that are needed for that process, too—a family of closely related molecules that they named the BIRDs. There were hints that the BIRDs might help corral SHORT-ROOT, which is produced in the vascular tissue, into the right layer of a developing root to exert its effects, but Benfey and his colleagues wanted to understand their roles more completely.

Miguel A. Moreno-Risueno, a postdoctoral researcher in Benfey's lab who now heads a lab at the Center for Plant Biotechology and Genomics in Madrid, carried out a series of experiments in which he examined what happened to growing roots when they were missing certain BIRD proteins. He found that roots that were missing just one BIRD protein developed with few defects. But eliminating combinations of the factors had some dramatic effects.

In plants without BLUEJAY, JACKDAW, and SCARECROW, ground tissue developed normally in the embryo, but stopped developing as the plant matured. The stem cell that gives rise to the ground tissue and the endodermis is lost. "We don't know of any other instance in plants where a combination of mutant factors causes a tissue to disappear entirely," Benfey says. "It's a bit like losing a liver. This tissue is a functioning, key part to the plant, and yet it's completely gone."

The BIRDs are transcription factors, meaning they exert their effects by controlling the activity of target genes. Benfey's team found that when the BIRDs are missing, cells fail to switch on genes that are important for ground tissue identity.

Taken together, the team's analysis shows that the BIRDs work in a complementary fashion—rather than a linear pathway—to control key aspects of ground tissue development in plant roots. "All of these factors are needed to get cortex and endodermis made," Benfey says. "They are involved in a three-step process: specifying the stem cell, getting the stem cell to divide correctly, and then getting the identity of their progeny to occur."

Benfey and his colleagues will continue to investigate the detailed steps that transform a stem cell into differentiated tissue in the plant root – gleaning new knowledge that will be critical for a fuller understanding of developmental biology.

More information: M. A. Moreno-Risueno et al. Transcriptional control of tissue formation throughout root development, *Science* (2015). DOI: 10.1126/science.aad1171

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