Open and Shut Case

Researchers are uncovering details of the molecules that control the development of stomatal pores on a leaf's surface.

IT'S NO WONDER that Dominique Bergmann talks about her *Arabidopsis* cells acting like toddlers and adolescents. Just the way a parent marks her kids' heights on a doorframe, she's spent her career charting plant cell development.

"How do you go from a naïve stem cell with all sorts of possibilities to being committed to a fate as a differentiated cell?" wonders Bergmann, an HHMI-GBMF investigator at Stanford University. "We don't have anywhere a whole record of how an organ is built from the beginning to the end."

But Bergmann has now made huge strides in tracking from start to finish the development of discrete multicellular structures on a leaf and in plotting the decisions their cells were making along the way. In an enterprising new study published April 6, 2015, in *Developmental Cell*, her laboratory tracked the shifting genetic programs of developing leaf cells as stem cells arise, differentiate, and form a complete structure.

The ambitious investigation was possible, in part, because the structure she studies is made up of just two specialized guard cells that form a pore on the leaf's surface. The so-called stoma, Greek for "mouth," allows plants to breathe in carbon dioxide and expel oxygen. Stomata formation unfolds simply and elegantly over two days on the leaf surface, where it can be watched in real time. That gave Bergmann an edge: in 2006 and 2007, her lab team identified the three transcription factors that orchestrate, in sequence, a leaf cell's entry into the stomatal lineage, its initial commitment to become a guard cell precursor, and its terminal differentiation into a guard cell.

When that sequence is disrupted by mutations in the transcription factors, the appearance of stomata on leaves is altered. SPEECHLESS (SPCH) and MUTE mutants sport no "mouths," while FAMA mutants, named for the Roman goddess of spreading rumors, produce an overabundance of dysfunctional mouths. These three molecules provided Bergmann with a handy way of isolating cells at each developmental stage, so that her team could document how changes in the cells' genetic programs might direct developmental decisions.

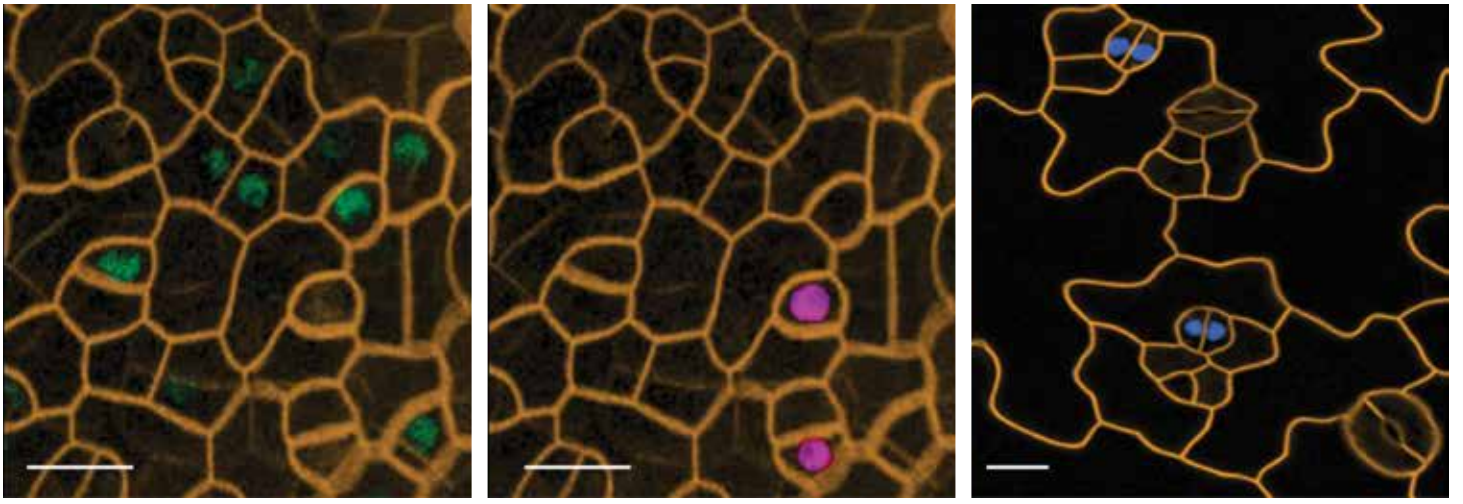
"If you think about the transition of the adolescent years into adulthood, a lot of things happen during that time," says Bergmann. "We wanted to capture this sort of growing up over developmental time. What genetic switch is flipping in these cells to say, 'I'm done with being a stem cell; I'm going to commit myself to something'?"

The team set out to use known cell-sorting techniques with fluorescently tagged versions of SPCH, MUTE, and FAMA, among other markers, to identify and separate the different developmental stages. However, those techniques were intended for free-floating mammalian immune cells, not plant cells locked together by cell walls.

Adapting techniques pioneered by HHMI-GBMF Investigator Philip Benfey, Bergmann's group was able to dissolve the cell walls, but they still had to amass enough of the fleeting intermediate stem cells to get an accurate readout of the cells' entire array of actively expressed genes. The sorting, combined with gene sequencing, revealed which genes were active at each transition in stomata development.

To see an animation of 24 hours of stomata development, go to hhmi.org/bulletin/spring-2015.

Images from a time series in stomata formation. Cells at different developmental stages are marked by nuclei labeled green (SPCH, stem-cell like), pink (MUTE, committed), or blue (FAMA, differentiating). The first two panels show the same group of cells at an early time point; in the third panel, taken a day later, cells are maturing. Scale bar in each is 5 microns.



Bergmann lab

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But, Bergmann admits, "It was a miserable experiment to do," requiring tens of thousands of seedlings to get at least 2,000 cells of each type.

Once her team had a list of the active genes present in each developmental stage of a stomatal cell's life, they compared their genetic profiles to those of plant cell types that had been characterized in other labs. They found that the earliest stem cells, tagged and sorted by SPCH, were highly variable and didn't quite match the profiles of any other plant cells out there.

"It was like a bunch of toddlers running around. There was great promise there, but you couldn't predict anything," says Bergmann. "We interpreted that as reflecting their pluripotency." (This observation complemented another recent finding by her lab team – that SPCH oscillations reset some leaf cells' identity to the stem-cell state, so the number of stomata on the leaf can adjust to environmental changes, such as in temperature or humidity.)

To study the next developmental stage, in which cells become the precursor guard mother cells, the team tagged and sorted cells using MUTE as a marker. At this stage, they found, cells had activated genes related to chromatin or DNA modification, which seemed to signal commitment. "They settled down – they are not turning on a bunch of functional proteins yet, but they are no longer pluripotent," Bergmann explains. "They've locked into that developmental path."

In the final, differentiated guard cell stage, the team saw some of those functional proteins come online, including active genes that encode sensory systems and ion channels that regulate the opening and closing of stomata.

Bergmann now feels she has an overarching view of the stoma's development – from a messy infancy full of potential, through an adolescence focused on choosing a path, to an adult phase with its fate locked down. "We see the cell go from a chaotic state, where it could do anything, to becoming more and more controlled," she says.

The keys to that process, it turns out, are the transcription factors that her lab identified: it's SPCH that opens the genetic doors to various nascent possibilities and FAMA that shuts those doors to lock in the cell's mature fate. *–Kendall Powell*