Unlikely pairs of DNA elements and regulator proteins make small plant stem cells destined to become stomata

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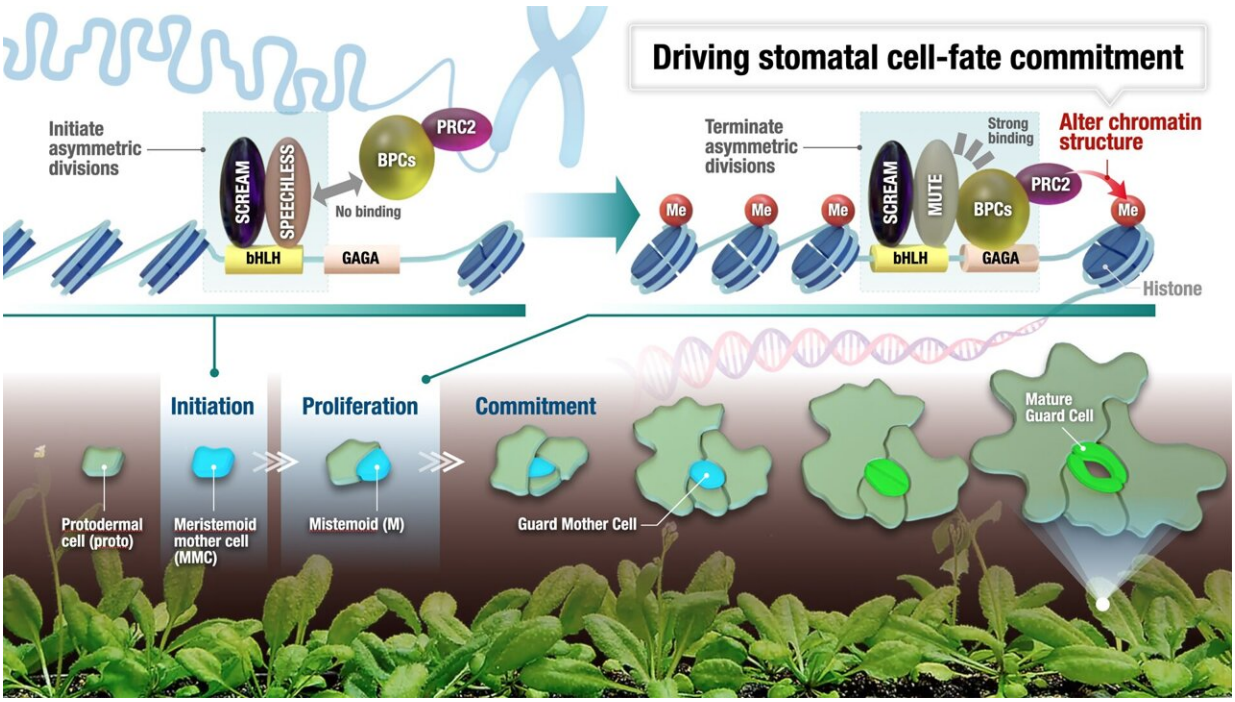

Schematic model of stem cell differentiation due to physical changes in genomic state caused by two different DNA sequences (cis-factors) and transcription factors (trans-factors) during stomatal development. Credit: Issey Takahashi

Researchers at Nagoya University in Japan, the University of Texas at Austin and the University of Washington have elucidated a mechanism

that makes tiny plant stem cells develop stomata, the cellular valves of plants that facilitate global carbon cycles.

Like our own cells, such as neurons and muscle fibers, plants have cells with special functionalities. One such cell type is a stoma (plural stomata)—a pair of guard cells that surround a pore for the efficient exchange of carbon dioxide and oxygen. The stomata adjusts the release of water vapors to prevent the plants from wilting. During the development of a dicot leaf, including that found in the model plant Arabidopsis, stem cells for stomata continue to emerge and each of them eventually becomes a stomatal guard cell.

Scientists know the identity of the master regulatory transcription factors of stomata, a type of protein that binds to DNA and regulates the expression of numerous genes to make stomata. They include SPEECHLESS and MUTE, two "sister" master regulators that sequentially initiate and terminate the stem cell state of stomata. However, a mystery remains: How can these master regulators coordinate with the actual genome state of stem cells to switch their fate?

In an article published in *Science Advances* on December 15, 2022, researchers reported the genome-wide atlas of the genome state (known as chromatin-accessibility) during stomatal development. In a nucleus of eukaryotic cells, genome DNA is bundled with histone proteins, a complex known as a chromatin. Transcription factors can access to the "open chromatin region," the place where the actions of gene expression are happening.

Using a technique called ATAC-sequencing, genome-wide profiling of accessible chromatins during stomatal development revealed that major reprogramming occurs at the point of stem cell proliferation to differentiation. The researchers also discovered that two DNA codes

(called cis-regulatory elements) are highly enriched in the early stomatal lineage: E-box, where transcription factors known as bHLH proteins bind, and GAGA-repeats, where transcription factors called BPCs bind in plants.

What is the significance of these two DNA codes? SPEECHLESS and MUTE, two sequentially acting master regulators, are bHLH proteins and they bind to these E-boxes. The researchers further discovered that MUTE, but not SPEECHLESS, strongly binds to BPCs, which bind to GAGA-repeats. Other scientists have shown that BPCs recruit enzymes that "tag" the repressive marks to chromatins. However, the current study revealed that during the differentiation of stem cells, MUTE binds with BPCs, then brings chromatin modifiers to establish repressive chromatin environment, thereby locking-in the genomic state to differentiation.

"We are very surprised and excited," said Professor Keiko Torii, the senior author. "Our genome-wide survey of chromatin accessibility tells us why it is important that the plants utilized sister master-regulators, SPEECHLESS and MUTE. They have similar but opposite roles—one initiates and maintains, and the other terminates the stomatal stem cell state.

"Now we know that only one of them—MUTE—can bring in BPCs to change the chromatin state. This means that two different classes of transcription factors and two different classes of DNA elements work together to lock in the fate of a plant cell. Our finding expands on how different cell types can be made."

More information: Yohei Takahashi et al, Stomatal CO 2 /bicarbonate sensor consists of two interacting protein kinases, Raf-like HT1 and non-kinase-activity requiring MPK12/MPK4, *Science Advances* (2022). DOI: 10.1126/sciadv.abq6161

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