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Stomatal differentiation: the beginning and the end

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Differentiation of stomata follows a series of stereotypical cell divisions and cell-state transitional steps specified by the master-regulatory transcription factors. The density and numbers of stomata are regulated by cell–cell signaling and flexibly modulated by environmental and physiological inputs. This review focuses on the latest breakthroughs in Arabidopsis elucidating the mechanisms behind the initiation of stomatal precursors, asymmetric cell division and stem cell behavior, and terminal differentiation of guard cells. I discuss new insights emerging from these studies: (i) competitive actions of signals and regulatory circuits initiating stomatal precursor pattern; (ii) a subcellular partitioning of signaling components determining the stomatal lineage stem-cell divisions; and (iii) epigenetic regulation maintaining the differentiated guard cell state.

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Introduction

Stomata are small epidermal valves that facilitate efficient gas exchange and control water loss in plants. Hence, density and number of stomata influence plant growth and productivity. Stomatal development has become an attractive system to study initiation, maintenance, and terminal differentiation of lineage-specific stem cells as well as regulation of tissue patterning. In Arabidopsis, stomatal lineage cells are specified by sequential activities of three basic-helix–loop–helix (bHLH) proteins, SPEECHLESS (SPCH), MUTE, and FAMA, that dimerize with partner bHLHs, SCREAM (SCRM)/ICE1 and SCRM2 (Figure 1a) [1,2]. SPCH, MUTE, and FAMA specify initiation and proliferation of stomatal stem cells (meristemoids mother cell [MMCs] and meristemoids), differentiation of guard mother cells (GMCs), and terminal differentiation of guard cells (GCs), respectively (Figure 1a) [1,2].

Cell–cell signaling enforces proper density and spacing of stomata. The molecular components of this signaling include secreted cysteine-rich peptides EPIDERMAL PATTERNING FACTOR (EPF)/EPF-LIKES, ERECTA-family leucine-rich repeat receptor kinases (LRR-RKs) and LRR-receptor-like protein TOO MANY MOUTHS (TMM), and downstream mitogen-activated protein kinase (MAPK) signaling cascade mediated by YODA (YDA), MKK4/5 and MPK3/6 [3–5]. Among these signaling components, EPF2 is primarily perceived by ERECTA to restrict initiation of stomatal cell lineages, while EPF1 is primarily perceived by ERECTA-LIKE1 (ERL1) to enforce stomatal spacing [3]. In addition, novel polarity proteins that execute the asymmetric cell division (ACD) within stomatal cell lineages have been identified [2,6].

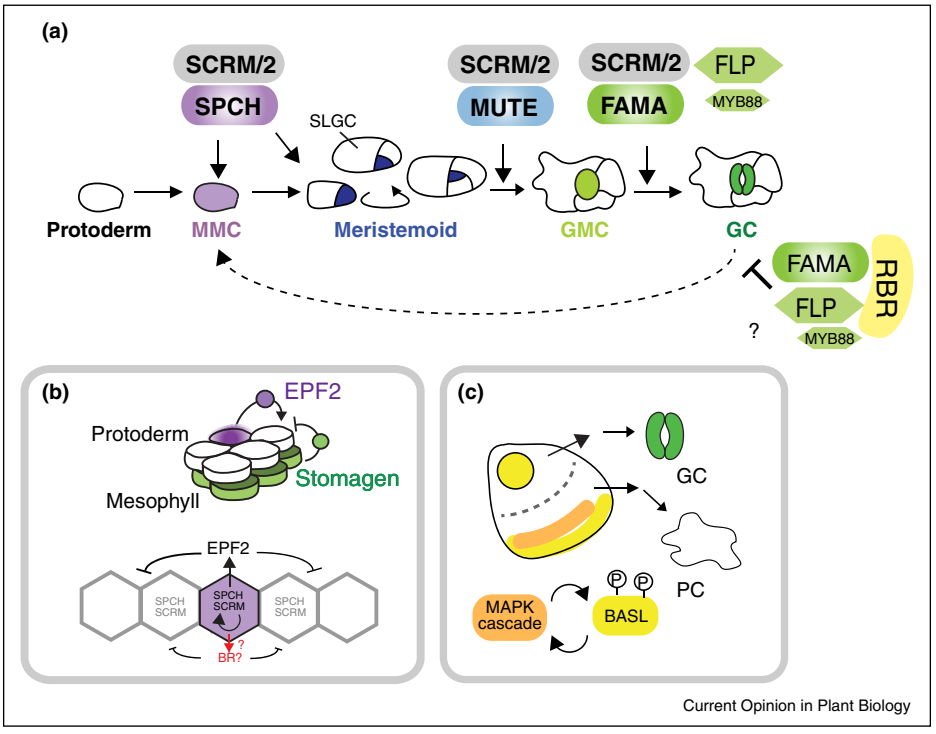
This review focuses on the latest breakthroughs in elucidating the molecular and cellular mechanisms behind the initiation of patterns, specification of cellular asymmetry, and maintenance of terminally differentiated state within stomatal cell lineages in Arabidopsis. By doing so, I wish to decipher the underlying principles in cell-type differentiation in plants. Many excellent reviews are available that describe the broader or specific topics in stomatal development not fully covered in this update [2,5,7–9].

Initial decision: regulatory circuits

During the development of photosynthetic organs, a subset of protodermal cells adopts an identity as MMC and undergoes an asymmetric entry division. SPCH and SCRMs (SCRM and SCRM2) specify this transitional step [10–12]. *SPCH*, *SCRM*, and *SCRM2* promoters are active in the entire protoderm [10,11,13••], indicating that all protodermal cells possess the potential to initiate stomatal development.

How can a subset of equipotential cells adopt stomatal-lineage identity, and what regulates their density and spatial distribution? Recent mathematical modeling and empirical studies unraveled that a positive feedback between SPCH and SCRM is crucial to 'lock in' the MMC fate, while two negative feedback loops that down-regulate the SPCH•SCRM module are necessary for proper spacing of stomatal initials (Figure 1b) [13••]. Chromatin immunoprecipitation (ChIP) experiments have demonstrated that *SCRM* is a direct downstream

Figure 1



Stomatal differentiation in Arabidopsis. **(a)** Diagram of cell-state transitional steps within stomatal cell lineages. A subset of protodermal cells (white) assumes MMC identity (purple) and executes an asymmetric entry division that creates a meristemoids (blue) and a sister cell, called SLGC (white). The meristemoids reiterates asymmetric amplifying division, but eventually differentiate into GMC (light green), which divides symmetrically once to form a stoma with differentiated GCs (green). SPCH, MUTE and FAMA direct sequential steps of transitions through heterodimerizing with SCRMs. FLP and its redundant paralog Myb88 also regulate the GMC symmetric division and GC differentiation. FAMA, FLP and Myb88 associate with RBR to maintain the terminally-differentiated GC state via epigenetically repressing the re-initiation of stomatal-lineage state (dotted line with arrowhead). Arrows indicate activation; T-bar indicates repression. Modified from [1]. **(b)** Stomatal lineage initiation. (Top) Stomagen peptides (green) secreted from developing mesophyll cells promote stomatal differentiation in the protoderm, while EPF2 peptides (purple) expressed in MMCs enforce proper patterning of stomatal precursor cells. The activities of the two antagonistic peptides fine-tunes stomatal patterning. (Bottom) Diagram of positive and negative feedback loops initiating stomatal cell lineage centered around the SPCH•SCRM module. EPF2-mediated negative feedback and an additional feedback, potentially mediated by BR pathway enforce stomatal patterning. Modified from [13]. **(c)** Diagram showing the unequal partitioning of MAPK activity by BASL. BASL (yellow) associates with YDA, a component of MAPK cascade (orange), and MPK3/6 phosphorylates BASL. Their positive feedback results in self-organized, polar localization of MAPK activity, which specifies SLGC and eventual pavement cell (PC) fate. BASL is localized in a nucleus in meristemoids, which results in low MAPK activity and eventual differentiation of guard cells (GC: green).

target of SPCH and that *SCRM* requires both SPCH and SCRM itself for protodermal expression in the cotyledons and leaves [13••,14••]. Moreover, SPCH and SCRMs directly induce the expression of *EPF2* and *TMM* [13–17]. Perception of EPF2 by TMM and ERECTA receptors inhibits the SPCH•SCRM module likely via MAP kinase-phosphorylation-mediated protein degradation [18,19], thereby completing the negative feedback loop.

The modeling approach revealed that an additional negative feedback loop, which is regulated by the SPCH•SCRM module but independent of EPF2, is necessary to recapitulate observed stomatal patterns. Brassinosteroid (BR) signaling is an attractive candidate for such a feedback loop (Figure 1b), since a downstream BR signaling component, BIN2, directly phosphorylates YDA and SPCH to regulate stomatal development [20,21]. Indeed, genome-wide ChIP-sequencing analysis of SPCH binding sites identified BR biosynthetic and signaling genes, such as *CPD*, *BIN2*, *BIM2*, and *BEH1/2/3/4* as SPCH direct targets [14••]. However the BR biosynthetic and signaling pathways are known to be under feedback regulation [22], which complicates the dissection of SPCH-modulation of the BR pathway. Likewise, it is not known in which cells within the protoderm BR is produced or whether the cell–cell movement of BR contributes to the initial patterning of stomatal lineage cells. Examining the BR actions at a cellular resolution is required to resolve such unanswered questions.

Signaling from down under: the very beginning?

SPCH specifies the initiation of the stomatal cell lineage. What then regulates *SPCH* expression? Normally, *SPCH*