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Spotlight

Speedy Grass Stomata: Emerging Molecular and Evolutionary Features

Stomata in most land plants are formed by a pair of guard cells, controlling the water loss and the carbon dioxide uptake. The development, patterning, and density of stomata are fundamental traits for stomatal function, contributing to plant growth and productivity (Pillitteri and Torii, 2012). The stomata of most plant species consist of two kidney-shaped guard cells, while stomata of grass species are formed by two dumbbell-shaped guard cells flanked by two subsidiary cells (Figure 1A). The four-celled stomatal complex in grasses may facilitate a fast response to environmental cues for efficient photosynthesis and water use, possibly through the rapid transport of ions and osmolytes between guard cells and subsidiary cells (see Jezek and Blatt, 2017; Chen et al., 2017 and references therein). Given that many grasses are agronomically important species as staple food, feed, and biofuel sources, it is vital to understand the molecular mechanisms of grass stomata development and patterning, and of membrane transport in guard cells and subsidiary cells, which govern the opening and closure of stomatal pores.

Our understanding of the genetic control of stomata development in the model grass species *Brachypodium distachyon* has been advanced by two recent groundbreaking studies by Raissig et al. (2016, 2017). The authors investigated the roles and functions of inducer of CBF expression (ICE)/SCREAM (SCRM) basic helix-loop-helix (bHLH) and SPEECHLESS (SPCH), MUTE, and FAMA-like bHLHs in the model grass *B. distachyon* by using mutagenesis, genome editing, and other emerging technologies. Their most exciting discovery is that BdMUTE acts as one of the key regulators for subsidiary cell formation and proper guard mother cell division. A 5-bp deletion in *BdMUTE* led to a failure in recruitment of subsidiary cells, and rendered stomata with only two guard cells in the *subsidiary cell identity defective* (*sid*) mutant, which could be complemented by *BdMUTEp*:*BdMUTE*. BdMUTE protein was detected in both guard cells and subsidiary cells, but intriguingly *BdMUTE* gene is expressed in guard mother cells, suggesting that BdMUTE protein moves from guard mother cells into subsidiary mother cells, probably via plasmodesmata. The loss of subsidiary cells in *sid* could not be rescued by the introduction of *BdMUTEp*:*AtMUTE*, suggesting that mobility is an intrinsic feature of BdMUTE protein and is essential for subsidiary cell patterning in grasses. In the model eudicot *Arabidopsis*, the development of *Arabidopsis* stomata depends on group Ia bHLH transcription factors—AtSPCH, AtMUTE, and AtFAMA—in heterodimeric association with bHLH group IIIb partners, AtICE1 and AtSCRM2 (Pillitteri and Torii, 2012; Chater et al., 2017). Although many key genes regulating stomatal development and patterning have been identified and extensively studied in *Arabidopsis* (Pillitteri and Torii, 2012; Chater et al., 2017), the discovery of grass-specific function of their homologous genes has major implications for plant and agricultural science and evolutionary biology.

Grass stomata are unique in nearly all aspects of their physiology and development compared with non-monocot plant stomata: they show faster stomatal responses, distinct morphological and structural features, and unique membrane transport and signaling systems. We hypothesized that the success of grass is likely attributed to the evolution of highly responsive stomata capable of maximizing productivity in rapidly changing environments; grass stomata harness the active turgor control mechanisms present in stomata of more ancient plant lineages, maximizing several morphological and developmental features to ensure rapid responses to environmental inputs (Chen et al., 2017 and references therein). This hypothesis is supported by the work on subsidiary cell-less *sid* mutant in Raissig et al. (2017). These authors found that the maximum pore area of stomata in *sid* mutant was only half of that in the wild-type, even when forced open by the toxin fusicoccin. The *sid* mutant showed slower stomatal responses to changing light intensities, and its stomata exhibited a reduced dynamic range of apertures compared with the wild-type. Most importantly, the *sid* mutant produced less biomass than the wild-type, linking BdMUTE-regulated subsidiary cell formation with impact on photosynthesis and biomass production in a grass species. The findings also suggest that engineering the properties of the subsidiary cell may allow for fine-tuning stomatal responses, which affect photosynthesis, water use efficiency, and crop yield.

The early acquisition of stomata is fundamentally important for the evolution of land plants, and many key genes encoding stomatal functions have been conserved since the first stomatal-bearing bryophytes (Chen et al., 2017 and references therein). Chater et al. (2016) showed that stomatal development in the moss *Physcomitrella patens* requires *PpSMF1* (*SPCH*, *MUTE*, and *FAMA-like*) and *PpSCRM1*, which are the orthologs of *SPCH*/*MUTE*/*FAMA* and *ICE1*/*SCRM2* in *Arabidopsis*, respectively. This suggests that the stomatal development may be evolutionarily conserved among the land plant lineages. This hypothesis is further supported by an evolutionary analysis of relevant proteins among 26 key plant and algae species (Figure 1B).

Although homologous genes of *SMF* and *ICE*/*SCRM* are mostly conserved from mosses to angiosperms with stomata, growing evidence supports the idea that their functions have evolved substantially (Figure 1C). In *P. patens*, the cellular process of stomatal development does not need asymmetric division to produce a meristemoid cell. Instead, PpSMF1 and PpSCRM1 are sufficient to initiate stomatal development (Chater et al., 2016). However, in *Arabidopsis*, AtSPCH is required to establish

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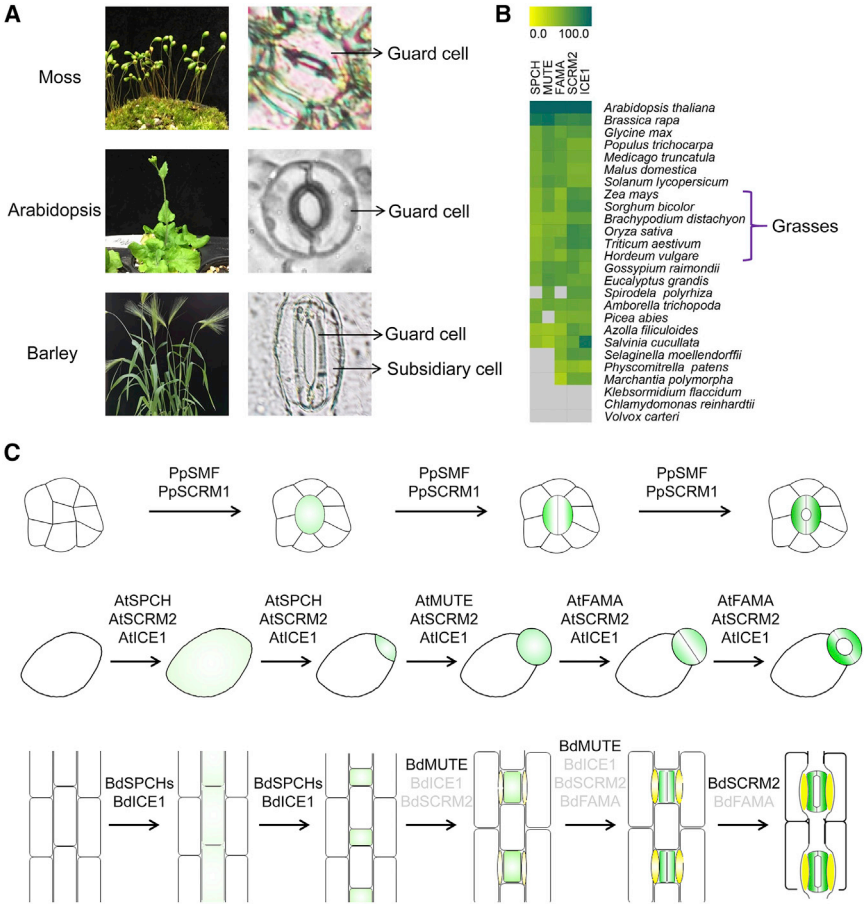

Figure 1. Evolutionary and Developmental Features for Speedy Grass Stomata.

(A) Images of plants and stomata of *Physcomitrella patens*, *Arabidopsis thaliana*, and *Hordeum vulgare*.

(B) Similarity heatmap for the evolution of stomatal development and patterning proteins in different species. Genesis software was used to estimate the similarity of proteins. Candidate protein sequences were selected by BLASTP searches using *Arabidopsis* sequences as the query with the criterion of E value $<10^{-5}$. Colored squares from zero (yellow) to 100% (green) indicate protein sequence similarity in each species compared with that in *Arabidopsis*. Gray squares indicate that no proteins were found that satisfied the selection criterion.

(C) Models of stomatal development in *P. patens*, *A. thaliana*, and *Brachypodium distachyon*. The roles and functions of SPCH, MUTE, and FAMA-like and ICE/SCRM bHLHs are divergent in different stages of stomatal development in moss, *Arabidopsis*, and *Brachypodium*. The proteins whose function has not been thoroughly studied are marked in gray. ICE1, inducer of CBF expression 1; SCRM2, scream2; SPCH, speechless; SMF, SPCH, MUTE, and FAMA-like.

stomatal lineage and drive asymmetric division, and AtMUTE helps to produce guard mother cells, followed by production of mature guard cells with AtFAMA (Ohashi-Ito and Bergmann, 2006; MacAlister et al., 2007; Pillitteri et al., 2007). The distinct stomatal morphology and patterning between monocots and dicots may be linked to the gene diversification and differential protein function. AtICE1 and AtSCRM2 act redundantly in stomatal development in *Arabidopsis*, whereas in *B. distachyon*, BdICE1 functions in establishing stomatal fate and BdSCRM2 is essential for differentiation of stomatal complexes (Raissig et al., 2016). Moreover, the foremost function of AtSPCH is to drive asymmetric division, but BdSPCHs were found to determine stomatal fate as BdSPCH2 has the potential in transdifferentiation of hair cells to stomata (MacAlister et al., 2007; Raissig et al., 2016).

In conclusion, a fundamental evolutionary innovation in *B. distachyon* stomatal development appears to be the mobility of BdMUTE protein, suggesting that BdMUTE might play a vital role in the formation of subsidiary cells in grasses species (Raissig et al., 2017). The findings by Raissig et al. (2016, 2017) and recent advances in the understanding of molecular evolution of stomatal development show that acquisition of these novel molecular and evolutionary mechanisms in grass stomata have resulted in faster and more efficient stomatal regulation in grass than in many other plant taxa. Utilizing genes of the bHLH family may well open an important route toward genetic engineering of major crops with "super stomata" to improve water use efficiency and yield. The breakthroughs in genome editing and other new technologies provide powerful tools for tackling these challenges to help meet the need imposed by the global climate change, booming world population, and declining arable land for food supply.

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