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Stomatal development in *Arabidopsis* and grasses: differences and commonalities

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ABSTRACT Stomata, found on the epidermis of all terrestrial plants, consist of two specialized cells called guard cells, which surround a tiny pore. Major advances have been made in our understanding of the genetic control of stomatal development in *Arabidopsis* and grasses. In *Arabidopsis*, three basic-helix-loop-helix (bHLH) genes control the successive steps that lead to stomatal formation. *SPEECHLESS (SPCH)* drives the cell division that initiates the stomatal cell lineage, *MUTE* induces the formation of the immediate stomatal precursor cell, and *FAMA* causes the stomatal precursor cell to divide into the two guard cells. Recent results demonstrate that these genes share functions with their grass homologs, and that *MUTE* is expressed later in development than its grass counterparts. Other differences in stomatal development between these two plant groups are exemplified by the *PANGLOSS1 (PAN1)* gene of maize. *PAN1*, which encodes a leucine-rich repeat receptor-like kinase with an inactive kinase domain, promotes polarization of the subsidiary mother cell and orients its cell division plane. Because such events do not exist in *Arabidopsis*, it is likely that the *PAN1*-like genes of *Arabidopsis* and *PAN1* are paralogs. Together, these results indicate that distinctions in the regulation of gene expression and protein function are both responsible for the divergence of stomatal development between *Arabidopsis* and grasses.

KEY WORDS: *Arabidopsis, bHLH, grasses, PAN1, stomata*

Introduction

The evolution of a hydrophobic cuticle that covers all aboveground plant surfaces and that is interrupted by stomatal pores allowed plants to survive on land (Edwards *et al.*, 1998). Stomatal pores, which seem to have arisen once during evolution, are present in the sporophyte generation of all land plants except for the liverworts (Raven, 2002). They are surrounded by two guard cells that swell following the influx of water from adjacent epidermal cells (Taiz and Zeiger, 2006). When the guard cells swell, the pore opens. Guard cells also contract upon water loss, which induces stomatal closure. The main function of stomata in vascular plants is to facilitate the capture of atmospheric CO_2 for photosynthesis, while keeping water loss to a minimum (Taiz and Zeiger, 2006).

It has been proposed that the stomata of vascular plants have never been replaced by other structures because they have been essential for adaptation to the terrestrial environment throughout land plant evolution. However, both the structure and the distribution of guard cells vary among plant groups. Kidney-shaped guard cells are characteristic of most dicots and non-graminaceous monocots, and dumbbell-shaped guard cells are typical in grasses and most other monocots (Weyers and Meidner, 1990). In grasses, leaves exhibit parallel venation, and stomata are arranged in linear files that make contact with non-stomatal-forming files. In contrast, dicots have reticulate venation, and stomata are scattered across the leaf surface (Hickey, 1979; Judd *et al.*, 1999; Fig. 1). These differences in stomatal patterns between grasses and dicots are a consequence of the developmental processes that give rise to stomata.

The cellular bases of stomatal development have been explored in numerous dicot species, with *Arabidopsis thaliana* being the best-studied system. Stomatal development in this model

Abbreviations used in this paper: BASL, BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE; bHLH, basic helix-loop-helix; EPF, EPIDERMAL PATTERNING FACTOR; PAN1, PANGLOSS1; SCREAM, SCRM; SPCH, SPEECHLESS, TMM, TOO MANY MOUTHS.

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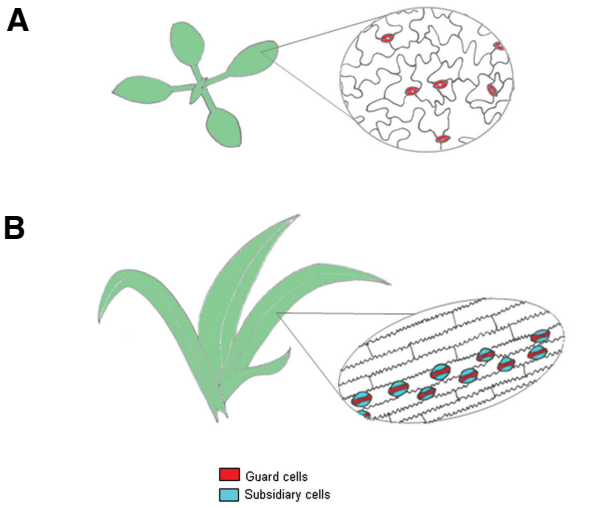

Fig. 1. The epidermis of *Arabidopsis* and rice leaves. (A) Arabidopsis. *Cells are randomly arranged, with stomata (paired guard cells) scattered across the surface.* **(B)** *Rice. Cells are arranged in linear files, with some files bearing stomata flanked by two subsidiary cells.*

plant starts with the division of an epidermal cell termed the meristemoid mother cell (Bergmann and Sack, 2007; Serna, 2007; Fig. 2A). This asymmetric cell division produces a small, triangular meristemoid cell and a larger cell. Meristemoids can divide asymmetrically in an inward spiral up to three times, always producing a larger cell and a smaller meristemoid that maintains its stem cell character. After these asymmetric cell divisions, the meristemoid loses its stem cell activity and acquires a subangular or rounded shape, which gives rise to the guard mother cell. The guard mother cell divides symmetrically to produce a pair of guard cells, which do not divide further. Although stomatal development occurs in a basipetal manner, with stomata maturing towards the leaf apex, new meristemoids arise among mature stomata, allowing intercalary growth.

In grasses, stomatal development starts with an asymmetric division, which yields the guard mother cell (Stebbins and Shah, 1960; Fig. 2B). Before it produces the paired guard cells, the lateral neighbors of the guard mother cell, termed subsidiary mother cells, divide asymmetrically to produce the two subsidiary cells. The guard mother cell then undergoes a symmetric cell division to generate paired guard cells, which are flanked by the subsidiary cells. In contrast to what is observed in dicots, stomatal development in grasses initiates at the leaf base, while cells expand and mature only near the apex.

During the last few years, an increasing number of genes regulating *Arabidopsis* stomatal development have been discovered (Peterson *et al*., 2010). Many of them function in a signalling cascade, which is initiated at the cell surface through the activation of the membrane receptors TOO MANY MOUTHS (TMM) and/or ERECTA family members by members of the EPIDERMAL PATTERNING FACTOR family (EPF1, EPF2, STOMAGEN/ EPFL9, CHALLAH/EPFL6), and/or a substrate processed proteolytically by the subtilase STOMATAL DENSITY AND DISTRIBUTION1 and transduced through cytoplasmic MAP kinases (YODA, MKK4/ MKK5, and MPK3/MPK6) towards the nucleus. These components negatively regulate the development of supernumerary stomata and enhance spacing among stomata. In the nucleus, these MAP kinases regulate the activity of the basic helix-loop-helix (bHLH) proteins SPEECHLESS (SPCH), MUTE, and FAMA, which act in concert with the bHLH-Leu zipper proteins SCREAM1 and SCREAM2 promoting stomatal development. The recent availability of genome sequences from grasses like rice (*Oryza sativa*) and maize (*Zea mays*) has permitted the analysis of genes that are homologs of *Arabidopsis* genes implicated in stomatal development (Liu *et al*., 2009), as well as the analysis of genes that most probably function exclusively in grasses (Cartwright *et al*., 2009). This review mainly focuses on the functions of *SPCH*, *MUTE*, *FAMA* and their homologs in grasses, highlighting the differences and commonalities of stomatal development in these two plant groups. The role of *PANGLOSS1* (*PAN1*) gene of maize, which encodes a leucine-rich repeat receptor-like kinase with an inactive kinase domain (Cartwright *et al*., 2009), is also highlighted.

Orthologs and paralogs of *SPCH*-like genes

In *Arabidopsis*, stomatal development starts with an asymmetric cell division. Mutations in the *Arabidopsis* gene *SPCH* that abolish its expression prevent the initiation of stomatal development and give rise to an epidermis consisting of only jigsaw puzzle-shaped pavement cells (MacAlister *et al*., 2007; Pillitteri *et al*., 2007). Thus, *SPCH*, which encodes a bHLH protein, is thought to drive the first asymmetric cell division that initiates stomatal development (MacAlister *et al*., 2007; Pillitteri *et al*., 2007). In support of this role, the overexpression of *SPCH* results in numerous extra cell divisions (MacAlister *et al*., 2007; Pillitteri *et al*., 2007). In addition to regulating the first asymmetric cell division that accounts for stomatal development, *SPCH* apparently maintains the stem cell activity of meristemoids (MacAlister *et al*., 2007): the missense mutation *spch-2*, which affects the carboxy terminus of the protein, reduces the number of cells entering the stomatal pathway and appears to trigger premature stomatal formation in the pedicel epidermis. *SPCH* promoter activity is observed in undifferentiated cells and persists in stomatal lineage cells, including guard cells (MacAlister *et al*., 2007; Pillitteri *et al*., 2007). However, the encoded protein is restricted to the early stages of the stomatal lineage, suggesting that the SPCH protein might be post-transcriptionally downregulated (MacAlister *et al*., 2007). These expression patterns are consistent with a role for *SPCH* in determining the entrance into the stomatal lineage and perhaps a later role in maintaining the stem cell activity of meristemoids.

In contrast to the single *SPCH* gene of *Arabidopsis*, poplar and *Ricinus*, Liu *et al*. found two *SPCH*-like genes in both *O. sativa* (*OsSPCH1* and *OsSPCH2*) and *Z. mays* (*ZmSPCH1* and *ZmSPCH2*). Like SPCH, OsSPCH2, ZmSPCH1 and ZmSPCH2 contain an N-terminal acidic region and, also like SPCH, the four grass homologs have a conserved mitogen-activated protein kinase (MAPK) target domain (Liu *et al*., 2009). The MAPK target domain of SPCH is phosphorylated by MAPKs (Lampard *et al*., 2008). Interestingly, the SPCH phosphorylation sites are conserved in the grass homologs (Lampard *et al*., 2008; Liu *et al*., 2009), suggesting that the *SPCH* function is also conserved in grasses. Certainly, in the case of *OsSPCH2*, this is supported by the observation that overexpression of *SPCH* under the control of

the *Cauliflower Mosaic Virus 35S* (*35S*) promoter leads to a phenotype identical to that induced by *OsSPCH2* overexpression, which consists of ectopic divisions in pavement cells (MacAlister *et al.*, 2007; Liu *et al.*, 2009). Furthermore, rice plants homozygous for the *osspch2-1* insertion, like *Arabidopsis* plants harboring the weak *spch-2* allele, have a reduced number of stomata (MacAlister *et al.*, 2007; Liu *et al.*, 2009). However, in contrast to the *spch-2* mutant, *osspch2-1* plants occasionally also develop stomatal patterning defects, and expression of *OsSPCH2* under the *SPCH* promoter fails to rescue the *Arabidopsis spch* phenotype (Liu *et al.*, 2009). Therefore, although *OsSPCH2* and *SPCH* appear to share some functions, their regulatory roles are not identical (Liu *et al.*, 2009). *OsSPCH2* controls patterning and it presumably has no role in the maintenance of meristemoid stem fate, given that rice meristemoids develop into guard mother cells without undergoing repeated rounds of asymmetric cell division. Remarkably, although *OsSPCH2* seems to control stomatal development, its expression has not been detected in leaves (Liu *et al.*, 2009). Overexpression of *OsSPCH1* by the *35S* promoter does not confer an apparent phenotype (Liu *et al.*, 2009), suggesting that the *OsSPCH1* function might have diverged from that of both *OsSPCH2* and *SPCH*. In support of this view, OsSPCH1 lacks the N-terminal acidic region that characterizes SPCH proteins (Liu *et al.*, 2009). In addition, the *OsSPCH1* transcript was not detected in leaves (Liu *et al.*, 2009). Mutation analysis of the *OsSPCH1* gene and cross-species tests will confirm (or annul) this apparent divergence.

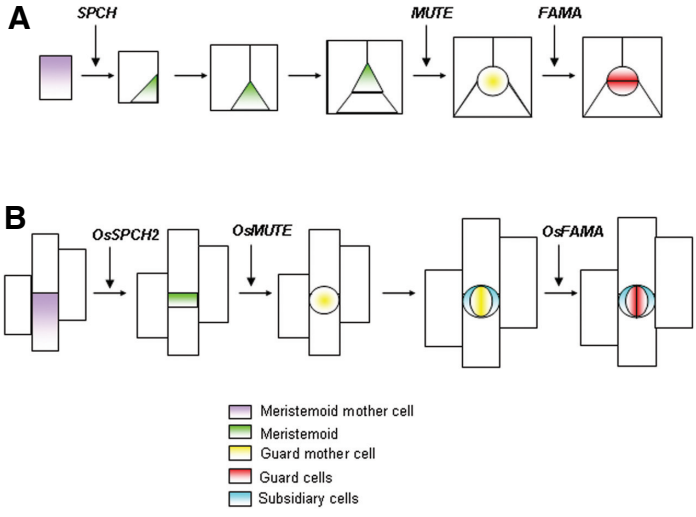

Fig. 2. **Stomatal development and *bHLH* genes. (A)** Arabidopsis. SPEECHLESS (SPCH) *initiates stomatal development by inducing the first asymmetric division, which gives rise to the first meristemoid. Two or three divisions after* SPCH, MUTE *represses the stem cell character of meristemoids and induces guard mother cell formation.* FAMA *then drives the symmetric division that gives rise to the two guard cells.* **(B)** *Rice.* OsSPCH2 *regulates the first asymmetric cell division in the stomatal lineage, which produces a meristemoid. Immediately afterwards,* OsMUTE *represses the stem cell character of the meristemoid and triggers guard mother cell differentiation. Consequently, meristemoids do not manifest stem cell properties. The lateral neighbors of the guard mother cell then divide asymmetrically to form stomatal subsidiary cells. Finally,* OsFAMA *causes the guard mother cell to divide, producing two guard cells. Adapted from (Serna, 2007; Liu* et al., *2009).*

In summary, these findings strongly suggest that *Arabidopsis SPCH* and rice *OsSPCH2* share some functions. Interestingly, *OsSPCH1*, which most probably originated as an *OsSPCH2* gene duplication, seems to have diverged from *OsSPCH2* and lost its function, and perhaps it acquired a new one. Certainly, when a gene duplicates, one copy generally retains the ancestral function, whereas the other copy is free to accumulate mutations (Ohno, 1970). Data on the role of *SPCH*-like genes in maize will reveal valuable information to extend the discussion on the molecular evolution of stomatal development to grasses.

MUTE-like genes may act earlier in grasses than in *Arabidopsis*

After a few cell divisions, meristemoids lose their stem cell identity and become guard mother cells, which then produce paired guard cells. *MUTE* plays an essential role in this transition by repressing the stem cell activity of meristemoids; the loss-of-function *mute-1* mutant does not develop stomata but forms meristemoids that abort after excessive asymmetric cell divisions (MacAlister *et al.*, 2007; Pillitteri *et al.*, 2007). The *MUTE* promoter is active in a subset of meristemoids, with residual activity in guard mother cells and immature stomata, whereas MUTE protein is restricted solely to a subset of meristemoids (MacAlister *et al.*, 2007; Pillitteri *et al.*, 2007). It is therefore probable that, in this subset of meristemoids, *MUTE* represses stem cell activity and induces guard mother cell formation. The fact that *MUTE* overexpression converts all epidermal cells into stomata is consistent with the function of this gene (MacAlister *et al.*, 2007; Pillitteri *et al.*, 2007).

Liu *et al.* found that, as in *Arabidopsis*, *MUTE*-like genes are present in grasses as single-copy genes. In addition, *OsMUTE* cDNA driven by the *MUTE* promoter partially rescues the *mute-1* phenotype by inducing the development of guard cells at the edges of leaves, while meristemoids remain arrested in the central regions of leaves (Liu *et al.*, 2009). Like *OsMUTE*, *ZmMUTE* expressed under the control of the *MUTE* promoter also complements the *Arabidopsis mute-1* mutant (Liu *et al.*, 2009). These lines of evidence suggest that the function of *MUTE*-like genes is conserved in grasses and *Arabidopsis*. Interestingly, the stomatal phenotype of *Arabidopsis* overexpressing either *ZmMUTE* or *OsMUTE* is similar to that caused by *MUTE* overexpression (at least when expression is at very high levels), in that all of the epidermal cells in cotyledons and leaves are guard cells (MacAlister *et al.*, 2007; Pillitteri *et al.*, 2007; Liu *et al.*, 2009). This finding also supports the designation of these genes as orthologs.

However, it remains undetermined whether *MUTE*-like genes abolish the stem cell character of meristemoids in grasses, and if so, how do they do this? Interestingly, grass meristemoids develop directly into guard mother cells without undergoing repeated rounds of asymmetric cell division. Grass *MUTE*-like genes may be expressed earlier in development than their *Arabidopsis* ortholog, that is, before the stem cell character of the meristemoids is manifested. Certainly, Liu *et al.* demonstrated that both *OsMUTE* and *ZmMUTE* are expressed when cell files are forming. Also consistent with this suggestion, *OsMUTE::GUS* expression in *Arabidopsis* is not restricted to a subset of meristemoids, but instead resembles the broad early expression pattern of the *SPCH::GUS* construct (MacAlister *et al.*, 2007;

Pillitteri *et al*., 2007; Liu *et al*., 2009). This result suggests that premature expression of *OsMUTE* (and *ZmMUTE*) may force guard mother cell formation after the first asymmetric cell division, preventing the stem cell activity of rice (and maize) meristemoids from persisting. If this is true, loss-of-function mutants of *OsMUTE* or *ZmMUTE* might be expected to have a phenotype similar to that of the loss-of-function *mute-1* mutant. Also, an obvious and testable corollary is that *MUTE* expressed under the control of the *OsMUTE* (or *ZmMUTE*) promoter in *Arabidopsis* should force meristemoids to develop directly into guard mother cells without undergoing repeated rounds of asymmetric cell division.

In contrast to both SPCH and FAMA, MUTE lacks both an N-terminal extension and a MAPK target domain. However, maize and rice MUTE orthologs exhibit multiple potential MAPK phosphorylation sites (Liu *et al*., 2009). It will be interesting to establish if there is any relationship between the presence of these MAPK phosphorylation sites in both OsMUTE and ZmMUTE, with respect to differences between the *MUTE* and *OsMUTE* (or *ZmMUTE*) expression patterns. Anyway, modifications in transcriptional regulation comprise an important component for the evolution of organismal phenotypes (Wray *et al*., 2003; Wray, 2003).

The role of *FAMA*-like genes is conserved in rice

In all plant species examined, the guard mother cell undergoes a symmetrical cell division to produce the two guard cells that form the stoma. In *Arabidopsis*, this transition from the guard mother cell to two fully differentiated guard cells is controlled by the *FAMA* gene, which encodes a member of the bHLH family of transcription factors (Ohashi-Ito and Bergmann, 2006). Plants homozygous for an insertion mutation in *FAMA* (*fama-1*), which lack the *FAMA* transcript, exhibit clusters of guard mother cells or immature guard cells instead of fully differentiated stomata (Ohashi-Ito and Bergmann, 2006). These clusters of small and narrow epidermal cells are referred to as tumors (Ohashi-Ito and Bergmann, 2006). Thus, *FAMA* stops the proliferation of guard mother cells, which prevents tumor formation, and triggers the formation of paired guard cells. The expression of *FAMA* is restricted to guard mother cells and differentiating guard cells, and the FAMA protein localizes to the nuclei of these cells (Ohashi-Ito and Bergmann, 2006).

Both maize and rice encode a single *FAMA* homolog, which are very similar (more than 90%) to the *Arabidopsis FAMA* sequence (Liu *et al*., 2009). Like FAMA, these protein homologs contain an N-terminal acidic domain (Liu *et al*., 2009) that is required to activate transcription (Ohashi-Ito and Bergmann, 2006). Liu *et al.* also showed that plants homozygous for an insertion mutation in the *OsFAMA* gene (*osfama-1*), which also lack *OsFAMA* transcripts, develop two guard mother cells or immature guard cells instead of fully differentiated stomata. In contrast to the *fama-1* mutation, the *osfama-1* allele does not cause numerous tumor-like cells to form in stomatal cell rows (Liu *et al*., 2009). Thus, *OsFAMA* controls stomatal differentiation but does not appear to prevent proliferation. In support of this interpretation, the heterologous expression of *OsFAMA* in *Arabidopsis* under the control of the *FAMA* promoter partially rescues the *fama-1* phenotype, producing stomata that mature but that are arranged in tumors. Two explanations may account for these observations: 1) *OsFAMA* may have lost (or *FAMA* may have gained) part of its function in restricting cell division, and 2) the *FAMA* and *OsFAMA* functions are fully conserved, and rice guard mother cells, but not *Arabidopsis* guard mother cells, have cell cycle control features that prevent cell divisions upstream of *OsFAMA*.

Overexpression of *OsFAMA* under the control of the *35S* promoter, like that of *FAMA*, induces the production of unpaired guard cells (Liu *et al*., 2009). Guard mother cells may develop into guard cells without undergoing a symmetrical division (Ohashi-Ito and Bergmann, 2006). Thus, the levels of both FAMA and OsFAMA may be essential for regulating cell division, with high levels repressing cell division and forcing guard mother cells to directly differentiate into guard cells (Ohashi-Ito and Bergmann, 2006).

Characterization of the *FAMA*-like gene function in rice suggests that the ability of *FAMA*-like genes to drive the transition from guard mother cells to fully differentiated guard cells is conserved, at least in *Arabidopsis* and rice. More data on the role of *FAMA*-like genes in maize and in other plant species are required to further understand the function of *FAMA*-like genes in grasses.

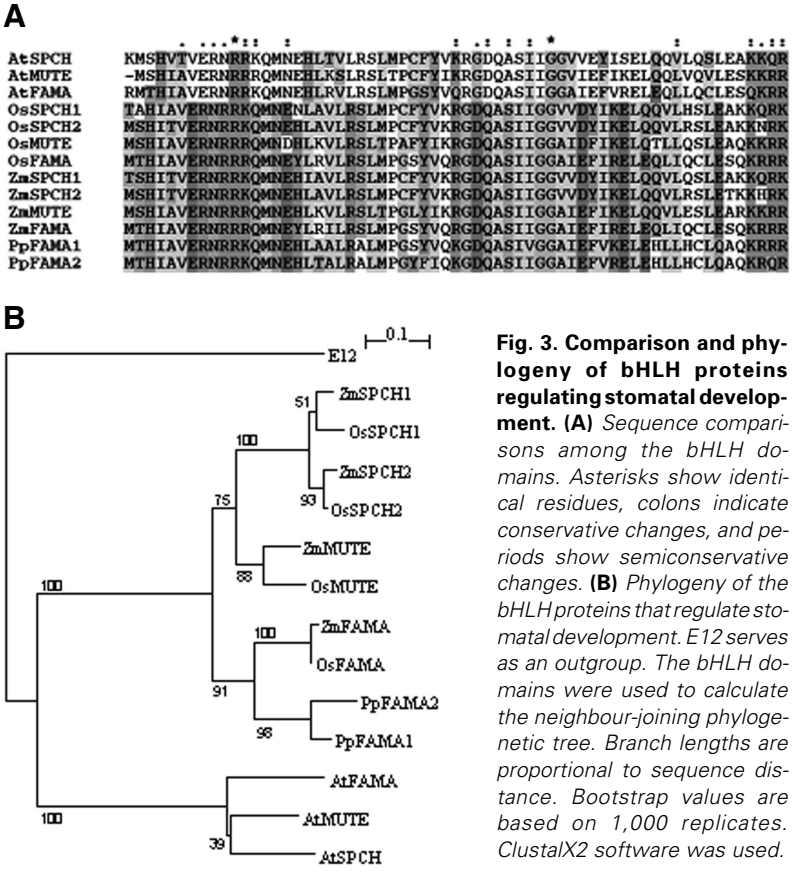

Fig. 3. Comparison and phylogeny of bHLH proteins regulating stomatal development. (A) *Sequence comparisons among the bHLH domains. Asterisks show identical residues, colons indicate conservative changes, and periods show semiconservative changes.* **(B)** *Phylogeny of the bHLH proteins that regulate stomatal development. E12 serves as an outgroup. The bHLH domains were used to calculate the neighbour-joining phylogenetic tree. Branch lengths are proportional to sequence distance. Bootstrap values are based on 1,000 replicates. ClustalX2 software was used.*

PAN1 and subsidiary cell formation in maize

In grasses, before guard mother cells produce paired guard cells, subsidiary mother cells polarize towards

the guard mother cell (Cho and Wick, 1990; Gallagher and Smith, 2000; Panteris *et al.*, 2006). This polarization is marked by the migration of the nucleus towards the guard mother cell and the formation of dense patches of cortical F-actin at the guard mother cell contact site. Mutations in two maize genes, *PAN1* and *PAN2*, cause defects in these two processes (Cartwright *et al.*, 2009). They also cause aberrant subsidiary mother cell divisions (Cartwright *et al.*, 2009). Thus, both *PAN1* and *PAN2* promote the polarization of the asymmetric subsidiary mother cell division and orient its cell division plane (Cartwright *et al.*, 2009). *PAN1*, which encodes a member of the leucine-rich repeat receptor-like kinase family, localizes within subsidiary mother cells to sites where these cells contact guard mother cells (Cartwright *et al.*, 2009). Analysis of PAN1-dependent protein phosphorylation revealed that PAN1 has an inactive kinase domain (Cartwright *et al.*, 2009). Consistent with these data, PAN1 lacks several amino acid residues that are critical for kinase activity (Hanks *et al.*, 1995; Cartwright *et al.*, 2009).

In *Arabidopsis*, the *TMM* gene also directs asymmetric cell division in stomatal development (Nadeau and Sack, 2002). TMM contains leucine-rich repeat and transmembrane domains, but it does not have a kinase domain (Nadeau and Sack, 2002). However, in contrast to *PAN1*, *TMM* does not promote the polarization of these asymmetric cell divisions, nor is it asymmetrically localized (Nadeau and Sack, 2002). Like *PAN1* (and *PAN2*), the *Arabidopsis BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE* (*BASL*) gene controls both the polarization of stomatal lineage cells and orients their asymmetric cell divisions (Dong *et al.*, 2009). Mutations in *BASL* disrupt the physical asymmetry of stomatal linage division and cause the production of more stomata (Dong *et al.*, 2009). *BASL*, which is expressed in stomatal lineage cells, encodes a novel protein for which homologs are found only in plants (Dong *et al.*, 2009). Also like PAN1, BASL exhibits a polar localization at the cell periphery in asymmetrically dividing cells (Cartwright *et al.*, 2009; Dong *et al.*, 2009). But unlike PAN1, BASL forms a crescent distal to the nucleus and it also accumulates in the nucleus (Cartwright *et al.*, 2009; Dong *et al.*, 2009).

TMM functions in a well-characterized signal transduction pathway (Bergmann and Sack, 2007). However, the up- and downstream components of the pathways in which *PAN1* and *BASL* operate are incomplete. The absence of PAN1 patches and the reduction of PAN1 levels in the *pan2* mutant suggest that *PAN1* functions downstream of *PAN2*, whose molecular nature remains unknown (Cartwright *et al.*, 2009). Future goals include unravelling the components of the pathways in which *PAN1* and *BASL* function, and identifying the *PAN2* gene product. Studies on the role of *PAN1*-like genes in *Arabidopsis* and in other grasses, and of *TMM*- and *BASL*-like genes in grasses, may reveal valuable information on the roles of these genes in the evolutionary context. Given that the asymmetric cell divisions that produce the subsidiary cells in grasses do not exist in *Arabidopsis*, it is likely that the *PAN1* and *PAN1*-like genes of *Arabidopsis* are paralogs.

Concluding remarks

Classification of plant bHLH proteins has allowed inferring that members of the same subfamily usually play redundant roles (Pires and Dolan, 2010). Strikingly, Pires and Dolan (2010) found that members of subfamily Ia proteins, which include SPCH, MUTE, FAMA, OsMUTE, OsFAMA and OsSPCH2, play non-overlapping roles. An attractive hypothesis considers that the *bHLH* genes encoding these proteins might have derived from a single *bHLH* gene in the ancestor of land plants, which would trigger the first asymmetric cell division and immediately guard cells formation. Interestingly, the moss *Physcomitrella patens* does not have *SPCH*- or *MUTE*-like genes, but has two *FAMA*-like genes (Peterson *et al.*, 2010). Consistently, stomata in moss develop through a single asymmetric cell division to produce the guard mother cell, which then divides to produce the paired guard cells (Payne, 1979). Then, it is likely that *MUTE*- and *SPCH*-like genes arose from duplications and divergences of *FAMA*-like genes, which allowed an increase in the complexity of stomatal development. In agreement with this view, the bHLH proteins of *Arabidopsis* and grasses fall into different evolutionary lineages (Fig. 3). Interestingly, FAMA is less similar to ZmFAMA and OsFAMA than is PpFAMA1 and PpFAMA2 (Fig. 3), which supports that stomatal development of *Physcomitrella patens* is more similar to that of grasses than that of *Arabidopsis*.

Knowledge of the functions of *SPCH*, *MUTE* and *FAMA* in *Arabidopsis* has facilitated understanding the roles of their orthologs in grasses (Table 1). Differences in the expression of *MUTE*-like genes appear to have contributed to the divergent patterns of stomatal development between *Arabidopsis* and grasses. The presence of homologs of the *Arabidopsis* signalling genes (for example, *YODA* and *TMM*) in grasses (Peterson *et al.*, 2010) suggests that a similar signalling cascade guides stomatal development in both groups. Similarly, the presence in rice of homologs of the *Arabidopsis* bHLHs ICE1/SCREAM (SCRM) and SCRM2, which seem to control stomatal development through their physical interaction with SPCH, MUTE, and FAMA (Kanaoka *et al.*, 2008; Liu *et al.*, 2009), suggests that similar multimeric complexes trigger stomatal development in the two plant groups. In contrast, the development of trichomes in *Arabidopsis* and grasses most likely depends on different transcriptional regula-

TABLE 1

bHLH GENES REGULATING STOMATAL DEVELOPMENT IN _ARABIDOPSIS_ AND GRASSES

Gene name	Function	Reference
AtSPCH	Drives the first asymmetric cell division. Maintains the stem cell activity of Ms	MacAlister *et al.*, 2007; Pillitteri *et al.*, 2007
OsSPCH1	Plays no role in stomatal development. Its function might have diverged from that of both *OsSPCH2* and *AtSPCH*	Liu *et al.*, 2009
OsSPCH2	Drives the first asymmetric cell division. Controls stomatal patterning	Liu *et al.*, 2009
Zm SPCH1	Might play a similar role to *OsSPCH1*	Liu *et al.*, 2009
Zm SPCH2	Might play a similar role to *OsSPCH2*	Liu *et al.*, 2009
AtMUTE	Represses stem cell fate of Ms and promotes GMC formation	MacAlister *et al.*, 2007; Pillitteri *et al.*, 2007
OsMUTE	Forces GMC formation after the first asymmetric cell division	Liu *et al.*, 2009
ZmMUTE	Forces GMC formation after the first asymmetric cell division	Liu *et al.*, 2009
AtFAMA	Stops the proliferation of GMCs and drives the transition from the GMC to the GCs	Ohashi-Ito and Bergmann, 2006
OsFAMA	Drives the transition from the GMC to the GCs	Liu *et al.*, 2009
ZmFAMA	Might play a similar role to *OsFAMA*	Liu *et al.*, 2009

tory networks (Serna and Martin, 2006).

The acquisition of the *PAN1* function in grasses (or its hypothetical loss in *Arabidopsis*) may also have contributed to divergences in stomatal development between *Arabidopsis* and grasses. Determining whether the commonalities and differences relative to stomatal development extend beyond these plant groups is essential for an in-depth understanding of the molecular evolution of stomatal development. Some interesting models of the roles that these genes may have in plants other than *Arabidopsis* and grasses have been proposed (Peterson *et al*., 2010). Future work will determine if these models reflect reality.

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References

BERGMANN, D.C. and SACK, F.D. (2007). Stomatal development. *Annu Rev Plant Biol* 58: 163-181.

CARTWRIGHT, H.N., HUMPHRIES, J.A. and SMITH, L.G. (2009). PAN1: A receptor-like protein that promotes polarization of an asymmetric division in maize. *Science* 323: 649-651.

CHO, S.O. and WICK, S.M. (1990). Distribution and function of actin in the developing stomatal complex of winter rye (*Secale cereale* cv. Puma). *Protoplasma* 157: 154-164.

DONG, J., MACALISTER, C.A. and BERGMANN, D.C. (2009).nBASL controls asymmetric cell division in *Arabidopsis. Cell* 137: 1320-1330.

EDWARDS, D., KERP, H. and HASS, H. (1998). Stomata in early land plants: an anatomical and ecophysiological approach. *J Exp Bot* 49: 255-278.

GALLAGHER, K. and SMITH, L.G. (2000). Roles for polarity and nuclear determinants in specifying daughter cell fates after an asymmetric cell division in the maize leaf. *Curr Biol* 10: 1229-1232.

HANKS, S.K. and HUNTER, T. (1995). Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *FASEB J* 9: 576-596.

HICKEY, L. (1979). A revised classification of the architecture of dicotyledonous leaves. In *Anatomy of the dicotyledons* (Eds. C.R. Metcalfe and L. Chalk). Second Edition, Vol. 1. Clarendon Press, Oxford, pp. 25-39.

JUDD, W.W., CAMPELL, C.S., KELLOGG, E.A. and STEVENS, P.F. (Ed.) (1999). *Plant systematics: A phylogenetic approach.* Sinauer Associates, Sunderland, MA.

KANAOKA, M.M., PILLITTERI, L.J., FUJII, H., YOSHIDA, Y., BOGENSCHUTZ, N.L., TAKABAYASHI, J., ZHU, J.-K. and TORII, K.U. (2008). *SCREAM/ICE1* and *SCREAM2* specify three cell-state transitional steps leading to *Arabidopsis* stomatal differentiation. *Plant Cell* 20: 1775-1785.

LAMPARD, G.R., MACALISTER, C.A. and BERGMANN, D.C. (2008). *Arabidopsis* stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. *Science* 322: 1113-1116.

LIU, T., OHASHI-ITO, K. and BERGMANN, D.C. (2009). Orthologues of *Arabidopsis Thaliana* stomatal bHLH genes and regulation of stomatal development in grasses. *Development* 136: 2265-2276.

MACALISTER, C.A. *et al.* (2007). Transcription factor control of asymmetric cell divisions that establish the stomatal lineage. *Nature* 445: 537-540.

NADEAU, J.A. and SACK, F.D. (2002). Control of stomatal distribution on the *Arabidopsis* leaf surface. *Science* 296: 1697-1700.

OHASHI-ITO, K. and BERGMANN, D.C. (2006). *Arabidopsis* FAMA controls the final proliferation/differentiation switch during stomatal development. *Plant Cell* 18: 2493-2505.

OHNO, S. (Ed.) (1970). *Evolution by gene duplication*. Springer-Verlag.

PANTERIS, E., APOSTOLAKOS, P. and GALATIS, B. (2006). Cytoskeletal asymmetry in *Zea mays* subsidiary cell mother cells: a monopolar prophase microtubule half-spindle anchors the nucleus to its polar position. *Cell Motil Cytoskeleton* 63: 696-709.

PAYNE, W.W. (1979). Stomatal patterns in embryophytes - Their evolution, ontogeny and interpretation. *Taxon* 28: 117-132.

PETERSON, K.M., RYCHEL, A.L. and TORII, K.U. (2010). Out of the mouths of plants: the molecular basis of the evolution and diversity of stomatal development. *Plant Cell* 22: 296-306.

PILLITTERI, L.J., SLOAN, D.B., BOGENSCHUTZ, N.L. and TORII, K.U. (2007). Termination of asymmetric cell division and differentiation of stomata. *Nature* 445: 501-505.

PIRES, N. and DOLAN, L. (2010). Origin and diversification of basic-helix-loop-helix proteins in plants. *Mol Biol Evol* 27: 862-874.

RAVEN, J.A. (2002). Selection pressures on stomatal evolution. *New Phytol* 153: 371-386.

SERNA, L. and MARTIN, C. (2006). Trichomes: different regulatory networks lead to convergent structures. *Trends Plant Sci* 11: 276-280.

SERNA, L. (2007). bHLH proteins know when to make a stoma. *Trends Plant Sci* 12: 483-485.

STEBBINS, G.L. and SHAH, S.S. (1960). Developmental studies of cell differentiation in the epidermis of monocotyledons. I. Cytological features of stomatal development in the *Gramineae*. *Dev Biol* 2: 177-200.

TAIZ, L. and ZEIGER, E. (Ed.) (2006). *Plant Physiology*. Fourth Edition. Sinauer Associates, Inc.

WEYERS, J.D.B. and MEIDNER, H. (Ed.) (1990). *Methods in stomatal research*. Longman Scientific and Technical, Harlow.

WRAY, G.A., HAHN, M.W., ABOUHEIF, E., BALHOFF, J.P., PIZER, M., ROCKMAN, M.V. and ROMANO, L.A. (2003). The evolution of transcriptional regulation in eukaryotes. *Mol Biol Evol* 20: 1377-1419.

WRAY, G.A. (2003). Transcriptional regulation and the evolution of development. *Int J Dev Biol* 47: 675-684.

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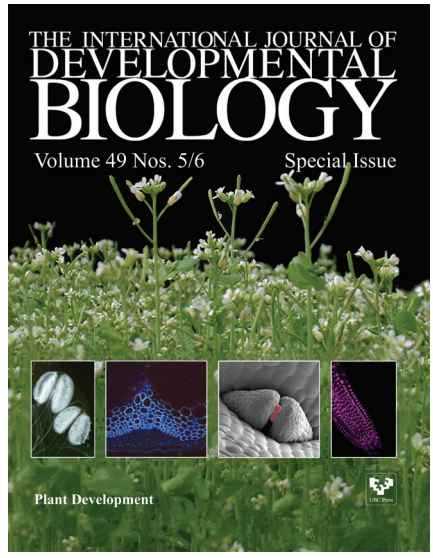

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