Emerging Parallels between Stomatal and Muscle Cell Lineages[1]

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Stomatal development provides a model for understanding the molecular basis of how cell lineages are established and how cells differentiate into functionally mature structures. This article describes recent advancements in understanding the role of basic helix-loop-helix (bHLH) proteins in stomatal lineage choice and differentiation in Arabidopsis. The emerging picture unravels that SPEECHLESS (SPCH), MUTE, and FAMA form heterodimers with SCREAM (SCRM)/ICE1 and SCRM2 to specify the sequential steps during stomatal development. Intriguingly, both key genes and mechanisms needed for stomatal development are also required for the formation of skeletal muscle in animals.

STEPS IN STOMATAL DEVELOPMENT

Stomata are epidermal structures that occur in most aerial organs of all terrestrial plants. They consist of two guard cells that delimit a pore and play an essential role in establishing adequate gas exchange between the plant and the atmosphere. The pore opening depends on changes in the turgor of the guard cells, and this in turn is controlled by the flow of water and ions between the guard cells and their neighboring adjacent epidermal cells (Taiz and Zeiger, 2006). Loss of guard cell turgor triggers stomatal closure, resulting in cessation of gas exchange, whereas gain in turgor induces the opposite effect.

In Arabidopsis (*Arabidopsis thaliana*), stomatal development starts with an asymmetric cell division from an epidermal cell named the meristemoid mother cell (Bergmann and Sack, 2007; Fig. 1A). This cell division produces a small meristemoid with a triangular shape and a larger cell. Meristemoids can divide asymmetrically in an inward spiral up to three times, always yielding a larger cell and a smaller meristemoid that maintains its stem cell character. The meristemoid, after these asymmetric cell divisions, loses its stem cell activity and adopts a rounded shape, giving rise to the guard mother cell. The guard mother cell undergoes a symmetric cell division that produces the paired guard cells, which do not divide further. The larger cells that result from the asymmetric divisions and make contact with the stoma (or its precursor) can either assume meristemoid mother cell identity entering into the stomatal cell lineage or become pavement cells.

Although stomata are essential for the plant even under laboratory conditions, which makes it difficult to isolate stomata-specific mutants, a large number of genes have been cloned that enable the understanding of stomatal formation in a molecular context. These include genes that encode for extracellular molecules (Berger and Altmann, 2000; Hara et al., 2007), cell membrane components (Nadeau and Sack, 2002; Shpak et al., 2005), cytoplasmic factors (Bergmann et al., 2004; Kutter et al., 2007; Wang et al., 2007), and nuclear factors (Lai et al., 2005; Ohashi-Ito and Bergmann, 2006; MacAlister et al., 2007; Pillitteri et al., 2007; Kanaoka et al., 2008). This article discusses the role of genes encoding for bHLH nuclear proteins during stomatal development of Arabidopsis. Interestingly, the similarities found between stomatal and skeletal muscle development reinforce the idea of a common underlying regulatory mechanism guiding these fates.

SPCH, MUTE, AND FAMA

Three earlier studies have shown that three *bHLH* genes, *SPCH*, *MUTE*, and *FAMA*, act sequentially in stomatal development, from the early decision to enter into the stomatal cell lineage to the last step when stomata are formed (Fig. 1A; for review, see Barton, 2007; Gray, 2007; Pillitteri and Torii, 2007; Serna, 2007). Plants either lacking detectable *SPCH* transcripts (*spch-3* and *spch-4*) or encoding a truncated SPCH protein without the last seven amino acids (*spch-1*) do not form stomata and exhibit an epidermal tissue consisting of only jigsaw-puzzle-piece-shaped pavement cells (Fig. 2; MacAlister et al., 2007). The lack of stomatal lineage cells in these mutants suggests that *SPCH* controls the initiation of stomatal development (Fig. 1A; MacAlister et al., 2007). Supporting such a role, the number of cells that initiate stomatal development, although not forming stomata, increases when *SPCH* is overexpressed (MacAlister et al., 2007; Pillitteri et al., 2007). The mutant *spch-2*, which encodes

[1] This work was supported by the Communities Council of Castilla-La Mancha (grant no. PCI08–0041–1136) and the Ministry of Science and Innovation of Spain (grant no. BIO2008–02149).

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www.plantphysiol.org/cgi/doi/10.1104/pp.108.133090

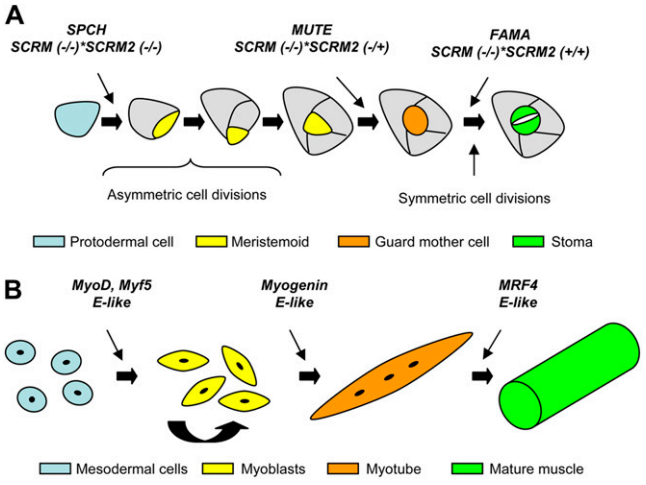


Figure 1. The role of *bHLH* genes in stomatal and muscle development. A, Stomatal development. *SPCH* starts stomatal development by inducing the first asymmetric division, which gives rise to the first meristemoid. Two or three divisions after the formation of the first meristemoid, *MUTE* drives the last asymmetric cell division, producing the guard mother cell. Then, *FAMA* regulates the symmetric division that gives rise to the two guard cells. *SCRM* and *SCRM2*, in a dosage-dependent manner, specify the actions of *SPCH*, *MUTE*, and *FAMA*. (Adapted from MacAlister et al. [2007], Pillitteri et al. [2007], Serna [2007], and Kanaoka et al. [2008].) B, Muscle development. *MyoD* and *Myf5* induce myoblast determination from mesodermal cells. Myoblasts remain in a proliferative state until *myogenin* instructs them to differentiate into myotubes. *MRF4* acts in late differentiation events, producing mature muscle. These MyoD family members function as heterodimers with the E-like proteins. (Adapted from Weintraub [1993] and Pillitteri and Torii [2007].)

a protein that differs from the wild type at the C terminus, exhibits a reduced number of stomata (MacAlister et al., 2007). The presence of stomata in *spch-2* enabled the unraveling of additional *SPCH* functions. Indeed, studies in the pedicel epidermis of this mutant showed that *SPCH*, in addition to controlling stomatal initiation, also maintains the stem cell activity of the meristemoids (MacAlister et al., 2007). Consistent with this dual function, the *SPCH* gene is broadly expressed, from undifferentiated epidermal cells to stomatal lineage cells (MacAlister et al., 2007).

MUTE also encodes a bHLH protein, which, in addition, is very similar in sequence to SPCH (Pillitteri et al., 2007). The loss-of-function *mute* mutant, with truncations in various positions within the bHLH domain, is completely devoid of stomata but develops meristemoids that abort after excessive asymmetric cell divisions (Fig. 2; MacAlister et al., 2007; Pillitteri et al., 2007). This suggests that *MUTE* represses stem cell activity of the meristemoids and induces guard mother cell formation (Fig. 1A). Consistently, the overexpression of *MUTE* converts all epidermal cells into stomata (MacAlister et al., 2007; Pillitteri et al., 2007). Both *MUTE* promoter activity and the MUTE protein localization are restricted to a subset of meristemoids, with very low activity in guard mother cells and developing stomata (MacAlister et al., 2007, Pillitteri et al., 2007). It is probable, therefore, that *MUTE* performs its function in those cells where it is expressed (Serna, 2007). MUTE also controls hydathode pore formation (Pillitteri et al., 2008).

FAMA was the first bHLH protein controlling stomatal development to be identified. It shares high sequence identity with SPCH and MUTE in the bHLH domain and C-terminal region (MacAlister et al., 2007; Pillitteri et al., 2007). Plants lacking detectable *FAMA* transcripts (*fama-1*) do not have mature stomata; instead, they develop groups of guard mother cells or immature guard cells (Fig. 2; Ohashi-Ito and Bergmann, 2006). These observations support the idea that *FAMA* induces guard mother cell division into two guard cells and that it promotes guard cell differentiation (Fig. 1A). Acknowledging its positive role in stomata formation, when *FAMA* is overexpressed nonstomatal cells convert directly to guard cells (Ohashi-Ito and Bergmann, 2006). Intriguingly, these guard cells are not organized into pairs; they develop from guard mother cells that become guard cells without undergoing a symmetrical division (Ohashi-Ito and Bergmann, 2006). One explanation for this finding is that *FAMA* levels are essential in the regulation of cell division versus differentiation, with high levels repressing cell division and forcing guard mother cells to differentiate directly into guard cells (Ohashi-Ito and Bergmann, 2006). *FAMA* promoter is induced in guard mother cells and young guard cells, and the FAMA protein localizes to the nucleus of these cells (Ohashi-Ito and Bergmann, 2006), which indicates that FAMA localizes in those cells where it exerts its action.

In summary, these *bHLH* genes have nonoverlapping but sequential roles in regulating stomatal development. At least *MUTE* and *FAMA* appear to play a major role in controlling stomata formation, because ectopic expression of any one in the nonstomatal cells results in a conversion of these cells to stomata or

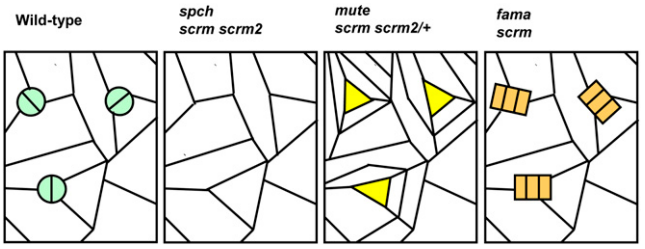


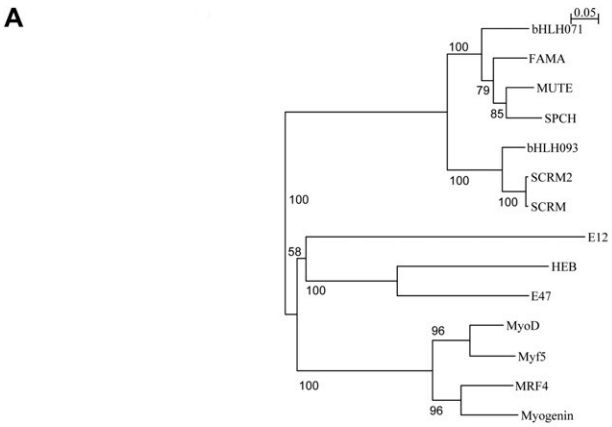
Figure 2. Stomatal phenotype of wild-type and mutant plants in genes encoding for bHLH proteins. Wild-type plants develop stomata that are spaced by intervening cells. Cells do not enter into the stomatal pathway in *spch* mutants. The *mute* mutant does not develop stomata but forms meristemoids that abort after excessive asymmetric cell divisions. *fama* lacks mature stomata and, instead, develops clusters of guard mother cells or young guard cells. The *scrm*, *scrm scrm2/+*, and *scrm scrm2* mutants phenocopy *fama*, *mute*, and *spch*, respectively. (Adapted from MacAlister et al. [2007] and Serna [2007].)

unpaired guard cells. Consequently, the cells must prevent their expression in the wrong cells or at inappropriate times or levels. Therefore, these genes necessarily have to be masterfully regulated.

SCRM/ICE1 AND SCRM2

The role for broadly expressed bHLH-Leu zipper proteins in stomatal development came from the analysis of gain-of-function mutants. Homozygous *scrm-D* mutants develop an epidermis composed of only stomata (Kanaoka et al., 2008), whereas heterozygous *scrm-D/+* plants exhibit a less severe phenotype with a high density of stomata making direct contact (Kanaoka et al., 2008). This strongly suggests that *SCRM* promotes stomatal development in a dose-dependent manner. Sequence analysis showed that the *SCRM* gene encodes the previously isolated bHLH-Leu zipper protein named ICE (Chinnusamy et al., 2003; Kanaoka et al., 2008). ICE is a regulator of freezing tolerance (Chinnusamy et al., 2003). The *scrm-D* phenotype is the result of a missense mutation within the KRAAM motif that replaces Arg-236 with His (Kanaoka et al., 2008). The truncated protein, similar to the wild-type one, localizes to the nucleus, which indicates that the *scrm-D* phenotype is not caused by a failure in the subcellular localization of the mutated SCRM protein (Kanaoka et al., 2008). Interestingly, an additional mutation destroying the DNA-binding ability restores the wild-type phenotype (Kanaoka et al., 2008). This strongly suggests that the *scrm-D* phenotype depends on the DNA binding. Equivalent amino acid substitution (Arg to His) within the KRAAM motif of the closely related SCRM paralog, SCRM2, gives rise to stomatal cluster formation (Kanaoka et al., 2008). Both SCRM and SCRM2 cluster in the same clade, which is distinct from the SPCH, MUTE, and FAMA clade (Fig. 3A). Similar to SCRM, SCRM2 also localizes to the cell nucleus (Kanaoka et al., 2008).

In contrast to *MUTE* and *FAMA*, both *SCRM* and *SCRM2* are broadly expressed in stomatal cell lineages, including meristemoids, guard mother cells, and differentiating guard cells (Kanaoka et al., 2008). Although *SPCH* and *SCRM/SCRM2* are broadly expressed in the epidermal tissue, their expression patterns are not completely overlapping. Certainly, *SPCH* is expressed in the entire protoderm (MacAlister et al., 2007; Pillitteri et al., 2007), being the first of the regulatory genes to be transcribed. Kanaoka et al.



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SPCH        KMSHVTVERNRRKQMNEHLTVLRSLMPCFYVKRGDQASIIGGVVEYISELQQVLQSLEAKKQRK
MUTE        -MSHIAVERNRRRQMNEHLKSLRSLTPCFYIKRGDQASIIGGVIEFIKELQQLVQVLESKKRRK
FAMA        RMTHIAVERNRRKQMNEHLRVLRSLMPGSYVQRGDQASIIGGAIEFVRELEQLLQCLESQKRRR
bHLH071     RMTHIAVERNRRRQMNQHLSVLRSLMPQPFAHKGDQASIVGGAIDFIKELEHKLLSLEAQKHHN
ICE/SCRM    PAKNLMAERRRRKKLNDRLYMLRSVVPK--ISKMDRASILGDAIDYLKELLQRINDLHNELEST
SCRM2       PAKNLMAERRRRKKLNDRLYMLRSVVPK--ISKMDRASILGDAIDYLKELLQRINDLHTELEST
bHLH093     PSKNLMAERRRRKRLNDRLSMLRSIVPK--ISKMDRTSILGDAIDYMKELLDKINKLQDEEQEL
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Figure 3. Phylogeny and comparison of bHLH proteins regulating stomatal development in Arabidopsis. A, Phylogeny of the bHLH proteins that control stomatal development. Myogenic bHLH proteins serve as an outgroup. SPCH, MUTE, and FAMA cluster in the same clade. bHLH093 clusters in the SCRM/SCRM2 clade. The bHLH domains were used to calculate the neighbor-joining phylogenetic tree using ClustalX2 software. Branch lengths are proportional to sequence distance. Bootstrap values are based on 1,000 replicates. The GenBank accession numbers are as follows (in parentheses): bHLH071 (NP_568666), FAMA (Q56YJ8), MUTE (ABI74926), SPCH (ABI26170), bHLH93 (NP_001078801), SCRM2 (ACA63683), SCRM (AAP14668), E12 human (CAC14267), HEB human (NP_996923), E47 human (NP_001129611), MyoD human (CAA40000), Myf5 human (NP_005584), MRF4 human (NP_002460), and myogenin human (NP_002470). B, Sequence comparisons among the bHLH domains. The H-E-R residues are shown in red. The basic region is shown in boldface type. Asterisks indicate identical residues, colons indicate conservative changes, and periods indicate semiconservative changes. Proteins were aligned using the ClustalW2 software.

(2008) have proposed that *SPCH* just might confer the competency to enter into the stomatal pathway and that *SCRM* and *SCRM2* might be required to initiate stomatal development.

Null alleles confirmed the positive role of these broadly expressed bHLH proteins in stomatal formation and also allowed in-depth insight into their specific roles. The loss-of-function mutation in *SCRM* induces the formation of groups of guard mother cells or immature guard cells instead of single and fully differentiated stomata, similar to those developing in *fama* (Fig. 2; Kanaoka et al., 2008). The epidermis of the *scrm scrm2* double loss-of-function mutant is indistinguishable from that of the *spch* mutant (Fig. 2; Kanaoka et al., 2008). Finally, the *scrm scrm2*/+ mutant exhibits an identical phenotype to *mute* (Fig. 2; Kanaoka et al., 2008). Together, these findings demonstrate that the dosage of these broadly expressed genes determines the successive steps that take place during stomatal development (Fig. 1A).

BROADLY EXPRESSED BHLH PROTEINS BIND TO CELL TYPE-SPECIFIC ONES

The bHLH family is defined by two functionally distinct regions (Littlewood and Evan, 1998). The helix-loop-helix region is located at the C-terminal end of the domain and is constituted mainly of hydrophobic residues. This region adopts a helix-loop-helix conformation in which two amphipathic α-helices are separated by an intervening loop of variable length, and it is required for dimerization with a partner of the same family. The basic region, with a large number of basic residues, is located at the N-terminal end, and it provides the contact points for an appropriate DNA target. In general, outside of the conserved bHLH domain, these proteins exhibit considerable sequence divergence (Atchley et al., 1999). Dimerization is a prerequisite for binding of bHLH-containing proteins to DNA (Murre et al., 1989a, 1989b; Davis et al., 1990). Some bHLH proteins form homodimers or restrict their heterodimerization activity to related members of the family (Littlewood and Evan, 1998). The core DNA sequence motif recognized by the bHLH proteins is a consensus hexanucleotide sequence known as the E-box (5′-CANNTG-3′) and identified for the first time in the immunoglobulin enhancers (Church et al., 1985).

Kanaoka et al. (2008) have made an in-depth study into the physical interaction among the bHLH proteins that control stomatal development. Bimolecular fluorescence complementation assays have shown that MUTE and FAMA strongly heterodimerize with SCRM and SCRM2. SPCH also associates with these broadly expressed proteins, but it shows only a weak interaction. In addition, with the exception of MUTE, these proteins do not form homodimers. The yeast two-hybrid system has confirmed that MUTE, FAMA, and SPCH physically associate with SCRM. The possible interactions between SCRM2 and cell type-specific bHLH factors in yeast have not been investigated. In summary, both yeast two-hybrid and bimolecular fluorescence complementation assays have shown that, in general, the cell type-specific bHLH proteins form heterodimers with the broadly expressed ones.

Yeast two-hybrid screening allowed the identification of two broadly expressed proteins, bHLH071 and bHLH093, as possible partners of FAMA (Ohashi-Ito and Bergmann, 2006). Bimolecular fluorescence complementation confirmed that these interactions take place in planta (Ohashi-Ito and Bergmann, 2006). It is unknown whether they interact with the remaining stomatogenic bHLH proteins. Loss-of-function mutations in *bHLH071* and *bHLH093* genes produce no obvious phenotype; however, the overexpression of either gene produces a weak *fama* phenotype (Ohashi-Ito and Bergmann, 2006). Either *bHLH71* or *bHLH093* overexpression may have titrated out an available pool of FAMA interfering with the formation (or function; see next paragraph) of biologically functional SCRM-FAMA or SCRM2-FAMA heterodimers.

It is likely that upon dimerization, these heterodimers with their basic regions recognize and bind to specific E-boxes and lead to transcriptional regulation of their target genes. Certainly, it is known that SCRM (ICE) binds to the CANNTG motif (Chinnusamy et al., 2003). In addition, SPCH, MUTE, and FAMA all contain the conserved residues H-E-R in their putative DNA-binding domains, which are present in proteins that bind DNA at the E-box (Fig. 3B; Shimizu et al., 1997). SCRM and SCRM2 contain a variant of such residues (N-E-R; Fig. 3B), which most probably also reflects binding to this conserved sequence. Both bHLH071 and bHLH93 also contain these conserved residues (H-E-R in bHLH071 and N-E-R in bHLH93; Fig. 3B). This suggests that in plants overexpressing either *bHLH71* or *bHLH093*, the hypothetical bHLH71-FAMA or bHLH93-FAMA heterodimers might successfully compete with SCRM-FAMA and/or SCRM2-FAMA for binding to specific E-boxes, preventing FAMA action.

PARALLELS BETWEEN STOMATAL AND MUSCLE DEVELOPMENT

Members of the bHLH family of transcription factors have also been shown to regulate the determination and differentiation of a variety of cell types, including skeletal muscle, neurons, and hematopoietic cells. Some parallels between muscle and stomatal development were previously highlighted (MacAlister et al., 2007; Pillitteri and Torii, 2007); however, the recent implication of SCRM and SCRM2 in stomatal development and their interaction with SPCH, MUTE, and FAMA reflect that a similar mechanism guides muscle and stomatal fate. In muscle development, an interplay of both tissue-specific bHLH regulators (MyoD family, which includes MyoD, myogenin, Myf5, and MRF4) and non-tissue-restricted bHLH

factors (E-like proteins) acts at multiple points to establish myoblast identity and control terminal differentiation (Fig. 1B; Lassar et al., 1991; Weintraub, 1993). In concert with E-like proteins, MyoD and Myf5 specify myoblast state, myogenin initiates myotube differentiation, and MRF4 acts in a later differentiation state producing mature muscle (Fig. 1B). Similar to *SPCH*, *MUTE*, and *FAMA*, the members of the *MyoD* family exhibit sequential expression patterns (for review, see Buckingham, 1992; Ohashi-Ito and Bergmann, 2006; MacAlister et al., 2007; Pillitteri et al., 2007). E-like proteins regulate wide varieties of developmental processes and pathogenesis. Similarly, SCRM controls not only stomatal development but also cold tolerance (Chinnusamy et al., 2003; Kanaoka et al., 2008). The four members of the MyoD family cluster in the same clade, which is distinct from the E-like protein clade (Fig. 3A).

The formation of heterodimers between regulators with restricted expression and those that exhibit a broad transcription not only affects stomatal development. MyoD family members also function predominantly as heterodimers with the E-like proteins, which include E12, E47, and HEB (Murre et al., 1989b; Hu et al., 1992). Myogenic bHLH factors can homodimerize, but the resulting complex can neither bind to nor activate muscle-specific genes (Murre et al., 1989a; Lassar et al., 1991). MUTE also forms homodimers (Kanaoka et al., 2008); the role of such complexes is unknown.

Myogenic heterodimers recognize and bind to the E-box consensus sequence (CANNTG) in gene muscle promoters and enhancers (Olson, 1990; Weintraub et al., 1991; Rudnicki and Jaenisch, 1995). E-boxes have been identified in promoters and enhancers of many skeletal muscle-specific structural genes, where they are required for activation by myogenic bHLH factors (Wentworth et al., 1991; Bessereau et al., 1993; Li and Capetanaki, 1994). As stated previously, it is known that SCRM binds to the CANNTG motif (Chinnusamy et al., 2003). In addition, SPCH, MUTE, FAMA, and bHLH071 all contain the conserved residues H-E-R in their putative DNA-binding domains (Fig. 3B), which are present in proteins that bind DNA at the E-box (Shimizu et al., 1997). Mutations in these residues in *FAMA* resulted in a nonfunctional protein (Ohashi-Ito and Bergmann, 2006). It seems that both myogenic and stomatogenic heterodimers bind to similar sequences, the E-boxes.

It is known that the activity of bHLH myogenic factors is regulated by phosphorylation/dephosphorylation events. For example, p38 mitogen-activated protein kinase phosphorylates MRF4, modulating its transcriptional activity (Suelves et al., 2004). The p38 mitogen-activated protein kinase also phosphorylates E47, which promotes MyoD/E4 association and muscle-specific transcription (Lluís et al., 2005). An elegant set of experiments showed that MPK3 and MPK4, which negatively regulate stomatal development (Wang et al., 2007), phosphorylate SPCH in vitro and modulate its activity in vivo (Lampard et al., 2008). So phosphorylation events regulate the activity of both myogenic and stomatogenic bHLH regulators.

In addition, both auto-regulatory and cross-regulatory interactions among bHLH factors have been demonstrated during muscle development (Braun et al., 1989; Thayer et al., 1989; Miner and Wold, 1990). For example, *MyoD* positively autoregulates its own expression and *myogenin* and *MyoD* regulate each other's expression (Thayer et al., 1989). Ohashi-Ito and Bergmann (2006) demonstrated that FAMA acts as a transcriptional activator. However, it seems that FAMA is not required to activate its own expression, as shown by the fact that the *FAMA* promoter is activated in *fama-1* cells (Ohashi-Ito and Bergmann, 2006). Reverse transcription-PCR analysis showed that MUTE is not required to activate its own transcription (Pillitteri et al., 2007). In contrast, SPCH positively autoregulates its own transcription (MacAlister et al., 2007; Pillitteri et al., 2007). Reverse transcription-PCR analysis also showed that SCRM activates its own expression (Kanaoka et al., 2008). SCRM2 also seems required to maintain its wild-type expression levels (Kanaoka et al., 2008). Interestingly, no *SCRM*::*GUS* expression has been detected in the *spch* epidermis (Kanaoka et al., 2008). In addition, the *scrm scrm2* double loss-of-function mutant does not express the *SPCH* gene (Kanaoka et al., 2008). This indicates that *SPCH* and *SCRM/SCRM2* regulate each other's expression. SPCH is also required to maintain the wild-type expression levels of *FAMA* and *MUTE* (MacAlister et al., 2007; Pillitteri et al., 2007). However, at least MUTE is not required to activate *SPCH* expression (Pillitteri et al., 2007). SCRM and SCRM2 are also required to activate both *MUTE* and *FAMA* expression (Kanaoka et al., 2008).

Furthermore, the forced expression of any of four *bHLH* genes from the *MyoD* family induces muscle differentiation in the transfected cell (Emerson, 1990; Weintraub et al., 1991; Buckingham, 1992; Weintraub, 1993; Olson and Klein, 1994), much as the ectopic expression of either *MUTE* or *FAMA* induces stomata or guard cell formation (Ohashi-Ito and Bergmann, 2006; MacAlister et al., 2007; Pillitteri et al., 2007). In contrast, ectopic expression of *SPCH* increases the number of cells that initiate stomatal development, although they seem to arrest their development because stomata are not formed (MacAlister et al., 2007; Pillitteri et al., 2007).

Myoblast differentiation or division is dictated by a balance of opposing signals controlled by myogenic bHLH proteins and peptide growth factors. The forced expression at high levels of the myogenic factors can induce cell cycle withdrawal and initiate myogenesis (Crescenzi et al., 1990; Sorrentino et al., 1990). This is similar to those occurring in plants overexpressing *FAMA*, which, in addition to activating guard cell formation, also play a role in regulating the exit from the cell cycle (Ohashi-Ito and Bergmann, 2006). It is likely then that these bHLH proteins modulate cell

cycle regulatory protein activity in a similar fashion in both animal and plant cells.

In spite of these strong similarities between these two systems, some differences have been found. Although myogenic bHLH factors are functionally interchangeable in gain-of-function studies (Wang and Jaenisch, 1997; Zhu and Miller, 1997), neither the expression of *FAMA* nor the expression of *MUTE* from the *SPCH* promoter substituted for SPCH function.

CONCLUDING REMARKS

Several recent findings have shown that myogenesis and stomatal development share not only a set of similar *bHLH* genes but also a common underlying mechanism. An intriguing property of the myogenic factors is their ability to self-regulate and activate the expression of other myogenic factors. Such regulatory interactions seem to reinforce the decision to differentiate and confer stability to the phenotype. An important challenge for the future will be to complete the possible cross-regulation and auto-regulation among the bHLH factors that guide stomatal development. An understanding of how these regulators control the cell cycle machinery ultimately to establish stomatal differentiation will also be important. The study of these problems will allow the similarities between muscle and stomatal development to be extended and, most importantly, some differences to be unraveled.

Received November 25, 2008; accepted January 27, 2009; published February 6, 2009.

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