Current Biology 19, 864–869, May 26, 2009 ©2009 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2009.03.069

Report

The Signaling Peptide EPF2 Controls Asymmetric Cell Divisions during Stomatal Development

Lee Hunt[1] and Julie E. Gray[1,*]
[1]Department of Molecular Biology and Biotechnology
University of Sheffield
Firth Court, Western Bank
Sheffield S10 2TN
UK

Summary

Stomata are pores in the plant epidermis that control carbon dioxide uptake and water loss. They are major regulators of global carbon and water cycles [1]. Several signaling components that regulate stomatal development have been characterized. These include a putative secretory peptide EPF1, LRR receptor components TMM and ER, and a peptidase SDD1 [2–4]. We have identified EPF2, a peptide related to EPF1 that is expressed in proliferating cells of the stomatal lineage, known as meristemoids, and in guard mother cells, the progenitors of stomata. EPF2 expression during leaf development affects stomatal density on the mature leaf. In the absence of EPF2, excessive numbers of cells enter the stomatal lineage and produce numerous small epidermal cells that express stomatal lineage reporter genes, whereas plants overexpressing EPF2 produce virtually no stomata. Results from genetic experiments indicate that EPF2 regulates a different aspect of stomatal development to EPF1 and are consistent with EPF2 acting in a pathway to regulate stomatal density that involves ER and TMM, but not SDD1. We propose that *EPF2* is expressed earlier in leaf development than *EPF1* and is involved in determining the number of cells that enter, and remain in, the stomatal lineage.

Results and Discussion

During *Arabidopsis* leaf development, selected epidermal cells become competent to undergo an asymmetric division resulting in the formation of a meristemoid and a larger daughter cell known as a stomatal lineage ground cell (SLGC). SLGCs may develop into epidermal pavement cells but some retain the ability to divide asymmetrically and form meristemoids. Each meristemoid usually divides asymmetrically several times before forming a guard mother cell (GMC), which divides symmetrically to form two guard cells that surround the stomatal pore. Asymmetric divisions of cells adjacent to stomata or meristemoids are oriented to ensure that stomata are always separated by at least one pavement cell [2, 3]. Studies have identified a number of gene products that affect the divisions of cells of the stomatal lineage. These include a putative processing protease, SDD1 [5, 6], a leucine-rich repeat domain (LRR) receptor-like protein, TOO MANY MOUTHS (TMM) [7, 8], the ERECTA (ER) family of LRR receptor kinases [9], components of a mitogen-activated protein kinase (MAPK) cascade [10, 11], and several transcription factors such as SPEECHLESS (SPCH) and MUTE [7, 12–17]. From analysis of plants with altered expression of these genes, it appears that an extracellular signaling pathway involving secreted peptides, in combination with a LRR receptor complex, activates an intracellular MAPK cascade that inhibits stomatal development by restricting the formation and division of meristemoids via phosphorylation of the transcription factor SPCH [13, 16]. Until recently, the ER family, TMM, and SDD1 were the only upstream components of this pathway identified. A secreted peptide ligand was predicted that could be processed by SDD1 and that could activate an ER family-TMM receptor complex at the cell membrane to inhibit stomatal development [5, 6]. The *Arabidopsis* genome encodes numerous predicted secretory peptides with unknown functions that could potentially fulfill this role [18]. Hara et al. [4] manipulated the expression of 153-peptide-encoding genes and found that the constitutive overexpression of *EPIDERMAL PATTERNING FACTOR 1* (*EPF1*) results in the inhibition of stomatal development. T-DNA disruption of the *EPF1* gene results in the breakdown of the "one-cell-spacing rule," thereby allowing stomata to form adjacent to one another in pairs that do not occur in wild-type plants. Genetic analysis showed EPF1 to act in the same pathway as the ER family of receptor kinases and TMM but suggested that EPF1 acts independently of the putative processing protease SDD1 to regulate stomatal development and clustering.

We have identified a putative *Arabidopsis* peptide encoded by *At1g34245* that has homology to EPF1. We have designated this peptide EPF2. Alignment of the predicted open reading frames revealed extensive homology between these peptides (46% similarity, 34% identity; Figure 1A). *EPF2* encodes a predicted peptide of 120 amino acids, MW 13 kDa, with a putative N-terminal signal sequence of 30 amino acids and is therefore, like EPF1, expected to be secreted. We investigated whether EPF2 could be involved in stomatal development. First, we characterized the gene expression pattern directed by the *EPF2* putative promoter region. DNA upstream of the predicted *EPF2* translation start was fused to a β-*glucuronidase* (*GUS*) reporter gene and *pEPF2:GUS* expressed in *Arabidopsis* plants. GUS activity was detected in very young leaves and during leaf development became restricted to meristemoids and GMCs (Figures 1B–1E). *EPF2* expression appeared to occur earlier in stomatal development than *EPF1* expression, which occurs in meristemoids, GMCs, and guard cells [4] (see also Figure 3E). In developing leaves of the same stage, *pEPF2:GUS* was expressed predominantly at the proximal end of the leaf, whereas *EPF1* was expressed toward the distal leaf tip (Figure S1A available online), consistent with *EPF2* being expressed at an earlier stage in leaf development. Our findings are in line with available transcriptomics results indicating maximal expression of *EPF2* prior to that of *EPF1* in juvenile leaves and in the vegetative shoot apex (Figure S1B).

To explore the function of EPF2 in developing leaves, we obtained *Arabidopsis* mutants with T-DNA insertions in the coding region of *EPF2* and identified two independent homozygous mutant lines (Figure 2A). Both *epf2-1* and *epf2-2* seedlings showed no expression of the wild-type *EPF2* transcript (Figure S2B). *epf2* plants grew and developed apparently

*Correspondence: j.e.gray@sheffield.ac.uk

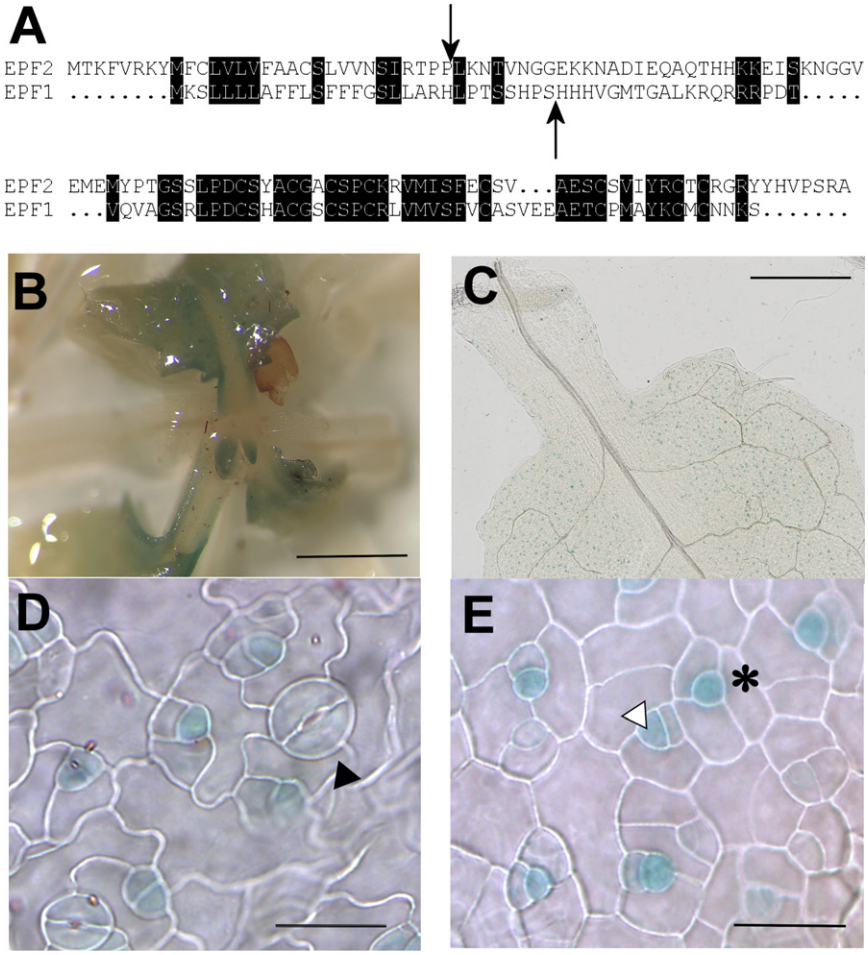

Figure 1. *EPF2* Encodes a Predicted Secretory Peptide that Is Expressed in Stomatal Lineage Cells of Developing Leaves

(A) Comparison of EPF1 and EPF2 predicted peptide sequences. Black boxes indicate similar and identical amino acids. Sequences were aligned with Multalin (http://bioinfo.genopole-toulouse.prd.fr/multalin/multalin.html) and displayed with Genedoc (http://www.nrbsc.org/gfx/genedoc/index.html). Arrows indicate signal peptide cleavage sites predicted by PSORT (http://psort.ims.u-tokyo.ac.jp/form.html).

(B–E) Histochemical staining of *pEPF2:GUS* (B) 3 week after germination seedling. Shown in (C) is a young developing leaf from seedling. Showing punctuate staining in cells of the stomatal lineage (D), (E) *pEPF2:GUS* expression is detected in early stomatal lineage cells including meristemoids (small triangular cells, example marked with white triangle) and GMCs (small oval cells, marked with asterisk) but not stomata (marked with black triangle) or neighboring cells of abaxial (C) and adaxial leaf surfaces (D). The scale bar represents 5 mm in (B), 100 μm in (C), and 10 μm in (D) and (E).

normally except that *epf2-2* was observed to have slightly narrower leaves. When examined microscopically, both *epf2-1* and *epf2-2* exhibited obvious defects in their leaf epidermal patterning that were indistinguishable between the two mutant alleles. Examination of the epidermis of leaves revealed the presence of numerous small epidermal cells and very few mature, tessellated, epidermal pavement cells in comparison to controls (Figures 2B–2E). The extra epidermal cells in *epf2* mutants appeared to result from additional epidermal cells undergoing divisions to enter the stomatal lineage together with extra asymmetric divisions of the neighboring SLGCs. Although the majority of these extra *epf2* cells did not differentiate into either pavement cells or guard cells, a significant increase in stomatal density was apparent in fully expanded leaves of both *epf2-1* (1.7-fold increase) and *epf2-2* (1.5-fold increase) (Figure 2G). In contrast to *epf1* mutants, clustered stomata were rare in *epf2-1 or epf2-2*.

Constitutive overexpression of EPF2 led to essentially the opposite phenotype of the *epf2* mutants, a significant reduction in stomatal development. We expressed *EPF2* with an epitope tag fused to the C terminus. Three independently transformed *p35S:EPF2-TAP* plants expressed a peptide of the predicted size (Figure S2C) and exhibited the same phenotype. The epidermis of leaves consisted almost entirely of epidermal pavement cells without arrested meristemoids or GMCs. It appeared that, in comparison to controls, fewer cells had entered the stomatal lineage or more cells had exited the stomatal lineage and differentiated into pavement cells (Figure 2F). *p35S:EPF2-TAP* leaves had a ~5-fold reduction in stomatal density (Figure 2G). Although *p35S:EPF2-TAP* plants had few stomata, those that were present had no apparent abnormalities. The *p35S:EPF2-TAP* plants appeared normal in other respects except that their growth was impaired, presumably because of their severe reduction in stomatal number.

To characterize the numerous small epidermal cells in *epf2* leaves, we transformed *epf2-1* with reporters for genes normally expressed in *Arabidopsis* stomatal lineage cells. The *TMM*, *SPCH*, *EPF1*, *SDD1*, and *MUTE* promoters direct reporter gene expression to stomatal lineage cells [3–5, 8, 13, 14]. In our experiments with developing *epf2-1* leaves transformed with *pTMM:GUS-GFP* or *pSPCH:GUS*, GUS activity and GFP fluorescence was observed in meristemoids, SLGCs, GMCs, guard cells, and small epidermal cells, indicating that the extra epidermal cells of *epf2* leaves are stomatal lineage cells resulting from divisions of meristematic cells (Figures 3A–3F). In contrast, no *pEPF1:GUS*, *pSDD:GUS*, or *pMUTE:GUS* was expressed in the additional small epidermal cells in the *epf2-1* background (Figures 3G–3L). Given that *pMUTE:GUS* expression is normally first detected in meristemoids that have undergone several divisions (and persists into GMCs and stomata) [14], these results suggest that the small cells of *epf2* were arrested as meristemoids prior to reaching this stage. The *pTMM:GUS-GFP* genetic marker was crossed into *p35S:EPF2-TAP* plants. Reporter gene expression was observed in fewer cells and at lower levels in plants overexpressing EPF2 than controls, suggesting that EPF2 may normally restrict entry to the stomatal lineage or perhaps promote exit to pavement cell fate (Figure S3).

We produced double-mutant plants by crossing *epf2-1* with other stomatal development mutants. Individually, *epf1* and *epf2* mutations cause increased stomatal densities (Figure 2G). In our experiments, very few paired stomata were observed in *epf2* mutants, and none in Col-0 controls,

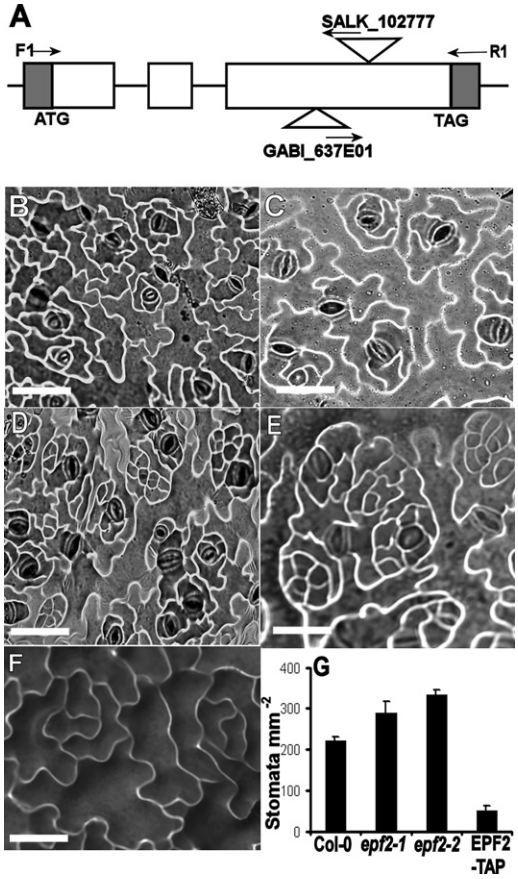

Figure 2. Plants with Disruptions in *EPF2* or Ectopically Overexpressing EPF2 Reveal a Role in Stomatal Development

(A) Diagram of position of T-DNA insertion sites in *EPF2* gene in *epf2-1* (SALK 102777) and *epf2-2* (GABI_637E01) mutants. Triangles indicate insertion sites, shaded areas indicate untranslated regions, and unshaded boxes represent coding regions. Arrows indicate PCR primer positions.

(B–F) DIC images of mature leaf abaxial epidermis impressions from Col-0 (B and C) and *epf2-1* (D and E) with 20× (B and D) and 40× (C and E) objective reveal the presence of extra-small epidermal cells in *epf2* mutants. As shown in (F), *p35S:EPF2-TAP* abaxial epidermis contains less stomata. The scale bar represents 60 μm in (B) and (D) and 30 μm in (C), (E), and (F).

(G) Abaxial stomatal densities of Col-0, *epf2-1*, *epf2-2*, and *p35S:EPF2-TAP* (EPF2-TAP) fully expanded leaves. Data are represented as mean ± SEM.

but as expected adjacent stomata were found in *epf1-1* [4] (Figures 4A–4C). *epf1-1 epf2-1* double-mutant plants showed an additive effect on stomatal density and stomatal pairing phenotypes in mature leaves over the single mutants (Figure 4D), suggesting that *EPF1* and *EPF2* control different aspects of stomatal development. In the experiment shown in Figure 4, *epf1-1* and *epf2-1* stomatal densities were both significantly higher than controls (1.2-fold and 1.3-fold) and *epf1-1 epf2-1* stomatal density was 1.9-fold higher than controls. *epf1-1 epf2-1* stomatal density was significantly greater than that of either *epf1-1* or *epf2-1* single mutants. The number of adjacent paired stomata was also significantly greater in *epf1-1 epf2-1* than that of either *epf1-1* (2.7-fold) or *epf2-1* (9.25-fold) (Figure 4F), indicating that any effect that *EPF2* has on stomatal pairing is also independent of *EPF1*.

sdd1 mutants, like *epf2* mutants, appear to have an increased number of cells entering the stomatal lineage. In *sdd1*, these cells develop into stomata and stomatal clustering occurs [5], whereas in *epf2-1*, the majority remain as small epidermal cells expressing stomatal lineage markers (Figure 3). *epf2-1 sdd1* double mutants showed additive stomatal density and pairing phenotypes over the single mutations (Figures 5A–5D). The lack of SDD1 in *epf2-1 sdd1* appeared to allow the additional cells entering the stomatal lineage, because of the lack of *EPF2*, to differentiate into stomata. *epf2-1* stomatal density was 1.2-fold greater, *sdd1* stomatal density was 2.4-fold greater, and *epf2-1 sdd1* was 4.4-fold greater than that of controls (all significant increases). *epf2-1 sdd1* stomatal density was significantly higher than that of either *epf2-1* (3.4-fold) or *sdd1* (1.85-fold) individual mutants (Figure 5I). The number of paired and clustered stomata was also much greater in *epf2-1 sdd1* than in either *epf2-1* or *sdd1* single mutants (Figure 5J). This additive *epf2-1 sdd1* double-mutant phenotype indicates that SDD1 is unlikely to process EPF2 and that they act independently.

In contrast to the additive effects of *epf2* in combination with either *sdd1* or *epf1*, the results from *epf2-1 tmm* and *epf2-1 er* double-mutant plants were less clear. There appeared to be a complex genetic interaction between *EPF2* and *TMM* because the *epf2-1 tmm* double-mutant phenotype was neither completely epistatic nor additive to the single-mutant phenotypes (Figure 5F). Although *epf2-1*, *tmm*, and *epf2-1 tmm* all had significantly increased mature leaf stomatal densities over controls, there were no significant differences in

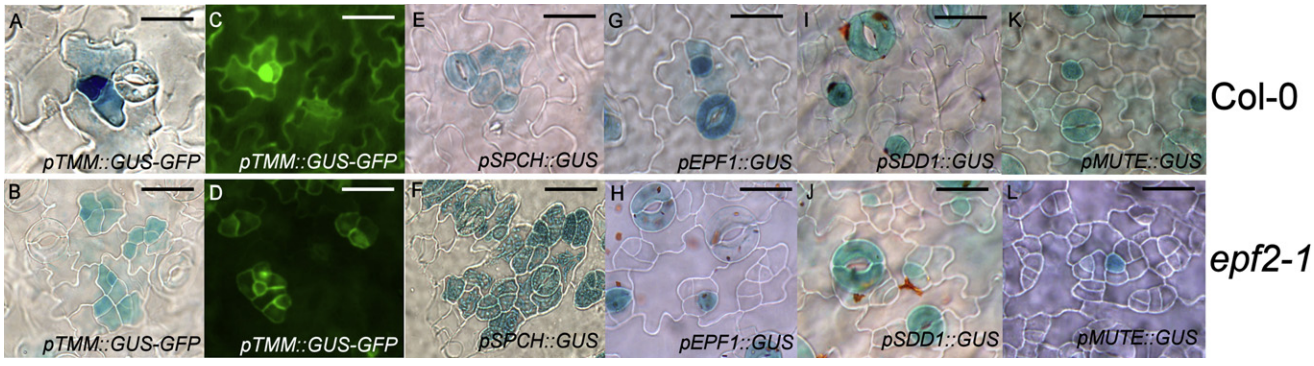

Figure 3. Additional Small *epf2-1* Epidermal Cells Express *TMM* and *SPCH* but Not *EPF1*, *SDD1*, or *MUTE* Stomatal Lineage Reporter Genes

GUS activity and GFP fluorescence of abaxial epidermal leaf surfaces from developing leaves of Col-0 and *epf2-1* seedlings expressing *pTMM:GUS-GFP* (A–D), *pSPCH:GUS* (E and F), *pEPF1:GUS* (G and H), *pSDD:GUS* (I and J) and *pMUTE:GUS* (K and L). Scale bars represent 20 μm.

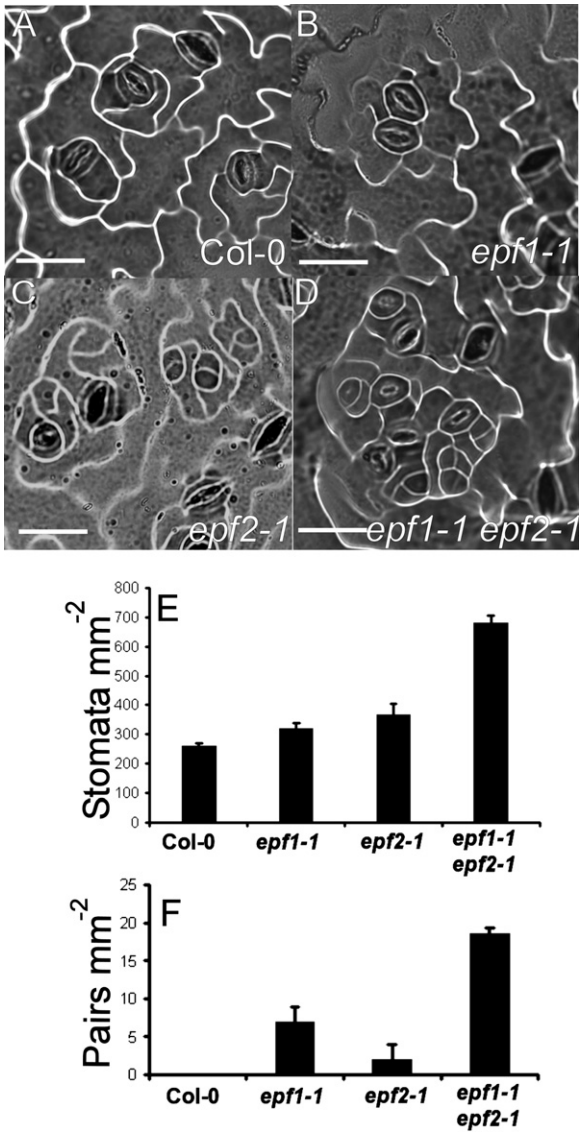

Figure 4. EPF1 and EPF2 Regulate Different Steps in Stomatal Development

DIC images of mature leaf abaxial epidermis impressions from Col-0 (A), *epf1-1* (B), *epf2-1* (C), and *epf1-1 epf2-1* (D). Scale bars represent 20 μm. (E) shows abaxial stomatal densities of Col-0, *epf1-1*, *epf2-1*, and *epf1-1 epf-2-1* fully expanded leaves. (F) shows densities of abaxial stomatal pairs from leaves as in (E). Data are represented as mean ± SEM.

stomatal densities among *epf2-1*, *tmm*, and *epf2-1 tmm*, indicating that *EPF2* and *TMM* may act in the same pathway to control stomatal density (Figure 5K). We also examined stem stomatal densities given that TMM acts as a positive regulator of stomatal development in stems. We observed very few stomata on *epf2-1 tmm* stems, suggesting that *tmm* may be epistatic to *epf2-1* in the control of stem stomatal density. However, closer examination of leaf epidermal patterning suggested complex effects for other phenotypic traits. Stomatal clustering (found in *tmm*) was clearly apparent in *epf2-1 tmm*, although at a much reduced frequency (Figure 5L). Thus, in leaves, EPF2 appears to be involved in the TMM-mediated pathway for controlling stomatal density and also has some role in the control of stomatal clustering. The extra-small stomatal lineage cells (found in *epf2-1*) were also apparent in *epf2-1 tmm*, suggesting that TMM is not necessary for the overproliferation of early stomatal lineage cells that we observed in *epf2-1*.

The *er* single mutant had arrested meristemoids in its leaf epidermis [14] but had no significant difference in stomatal density compared to controls (Figure 5G). The significantly increased stomatal density found in *epf2-1* (1.2 fold in this experiment) was not present in the *epf2-1 er* double mutant, suggesting that *er* may be epistatic to *epf2-1* with respect to stomatal density. The epidermal patterning phenotype of *epf2-1 er* was also most similar to *er*, containing arrested meristemoids rather than the overproliferation of small cells that is characteristic of *epf2-1* (Figure 5H). Thus, *er* appears to be epistatic to *epf2-1* with respect to proliferation of stomatal lineage cells. These results are consistent with *ER* acting in the same pathway as *EPF2* to control stomatal development.

In summary, the phenotypes of the double-mutant plants were consistent with EPF2 controlling the entry of cells into the stomatal lineage by a mechanism that involves ER but does not involve EPF1, SDD1, or TMM. Our analyses also indicated that EPF2 may control the number of cells that differentiate into stomata by a mechanism that is mediated by ER and TMM. Our results did not support a role for EPF2 in the control of stomatal clustering by EPF1 or SDD1 but indicated some role for EPF2 in the control of stomatal clustering by TMM. EPF2 appears to act early in stomatal development to regulate the asymmetric divisions that lead to meristemoid proliferation and differentiation (Figure S4). Our results suggest that EPF2 peptide, secreted from meristemoids, may affect the fate of neighboring epidermal cells by inhibiting meristemoid fate and promoting pavement cell fate, via a pathway that is independent of EPF1 and SDD1. They are also consistent with EPF2 activity being mediated, at least in part, by ER and TMM. These membrane proteins may act as receptor components in neighboring cells to perceive EPF2 peptide and initiate an intracellular response to regulate stomatal density by inhibiting meristemoid fate. Stomatal meristemoids are considered to have transient stem cell properties [3], and the inhibition of their proliferation by EPF2, perhaps in combination with an LRR-receptor complex, has parallels with the role of the secreted peptide CLAVATA3, which restricts stem cell accumulation in the shoot apical meristem [19, 20].

Experimental Procedures

Plant Material

epf2-1 (SALK_102777 [21]) and *epf2-2* (GABI_673E01 [22]), *sdd1* (GABI_627_D04 [4]), *tmm* (SALK_057932), and *er* (SALK-044110) seed stocks were obtained from NASC, and T-DNA insertion sites were verified by PCR (for primer sequences, see Table S1). All plants were in the *Arabidopsis thaliana* Col-0 background accession. These alleles of *tmm* and *er* have similar phenotypes in leaves and stems as those previously reported [7, 14].

Vector Construction and Plant Transformation

Approximately 2 kb 5′ of the predicted translation start site of *EPF1* (*At2g20875*), *EPF2* (*At1g34245*), *SDD1* (*At1g04110*), and MUTE (*At3g06120*) were PCR amplified with KOD polymerase (Merck Biosciences, Nottingham, UK). We recombined products into pENTR/D/TOPO and then with LR clonase II (Fisher Scientific, Leicester UK) into pKGWFSG7 binary vector [23] to create *pEPF1*:GUS and *pEPF2*:GUS, pHGWFSG7 to create *pMUTE:GUS*, and pGWB3 to generate *pSDD1*:GUS. The predicted coding region of *EPF2* was amplified from cDNA with KOD polymerase, recombined into pENTR/D/TOPO, and recombined into pCTAPi [24] with LR clonase II to create *p35S:EPF2-TAP*. Constructs were transformed into Agrobacterium C58 by electroporation and *Arabidopsis* plants were transformed by floral dipping [25]. Transformants were selected with kanamycin (*pEPF1*:GUS and

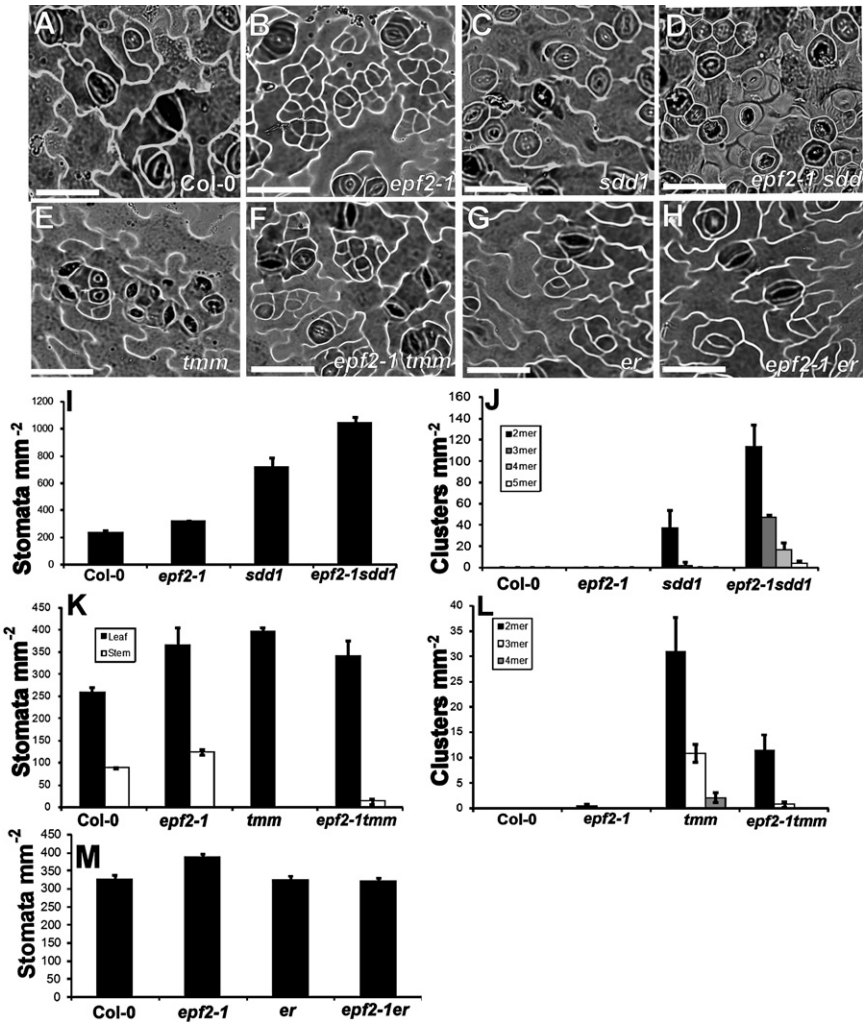

Figure 5. *EPF2* Genetically Interacts with *TMM* and *ER* but Is Independent of *SDD1*

(A–H) DIC images of mature leaf abaxial epidermis impressions from Col-0 (A), *epf2-1* (B), *sdd1* (C), *epf2-1 sdd1* (D), *tmm* (E), *epf2-1 tmm* (F), *er* (G), and *epf2-1 er* (H). Scale bars represent 20 μm.

(I, K, and M) Stomatal densities of abaxial surface of mature leaves (filled bars) or stems (open bars).

(J and L) Densities of stomatal clusters with two, three, four, or five adjacent stomata on abaxial surface of mature leaves. Data are represented as mean ± SEM.

pEPF2:GUS and *pSDD1*:GUS), hygromycin (*pSPCH:GUS, pTMM:GUS-GFP, pMUTE:GUS*), or Basta (Liberty; Agrevo, Cambridge UK) (*p35S:EPF2-TAP*).

Microscopy

For leaf epidermal cell counting, dental resin (Coltene Whaledent, Switzerland) was applied to the abaxial surfaces of fully expanded leaves and nail varnish peels were taken from set resin after removal of the leaf. Cell counts were taken from areas of three leaves from three separate plants of each genotype. To analyze stem epidermis, we cut secondary branches of inflorescences at the base and applied nail varnish. Varnish was transferred to tape and cell counts taken from three areas of three branches for at least three separate plants. Unpaired t tests were performed on data and $p < 0.05$ was regarded as significantly different. Histochemical staining for GUS activity was carried out on developing leaves of the same stage (~1 cm length) from 3- to 5-week-old T1 or T2 seedlings in 50 mM potassium phosphate, 1 mM potassium ferrocyanide, 1 mM potassium ferricyanide, 0.2% Triton X-100, 2 mM 5-bromo-4-chloro-3-indolyl-β-d-glucuronic acid, and 10 mM EDTA after vacuum infiltration at 37°C. Leaves were decolorized overnight with 70% (v/v) ethanol, washed 1 hr with 20% (v/v) ethanol, and cleared in 8:1:1 (w/v/v) chloral hydrate:water:glycerol. DIC images were captured with an Olympus BX51 microscope connected to a DP51 digital camera. For GFP, seedlings were mounted in water before imaging. Expression patterns shown were typical of at least two independently transformed plant lines.

Supplemental Data

Supplemental Data include four figures and one table and can be found with this article online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)00976-2.

Acknowledgments

pTMM:GFP-GUS and *pSPCH:GUS* were kindly provided by F. Sack (University of British Columbia) and D. Bergmann (Stanford University). pCTAPi was a gift from M. Fromm (University of Nebraska-Lincoln) and pGWB3 was a gift from T. Nakagawa (Shimane University).

Received: January 25, 2008
Revised: March 11, 2009
Accepted: March 26, 2009
Published online: April 23, 2009

References

1. Hetherington, A.M., and Woodward, F.I. (2003). The role of stomata in sensing and driving environmental change. Nature *424*, 901–908.
2. Bergmann, D.C., and Sack, F.D. (2007). Stomatal development. Annu. Rev. Plant Biol. *58*, 163–181.
3. Nadeau, J.A., and Sack, F.D. (2002). Stomatal development in *Arabidopsis*. In The Arabidopsis Book, C.R. Somerville and E.M. Meyerowitz, eds. (Rockville, MD: The American Society of Plant Biologists).
4. Hara, K., Kajita, R., Torii, K.U., Bergmann, D.C., and Kakimoto, T. (2007). The secretory peptide gene EPF1 enforces the stomatal one-cell-spacing rule. Genes Dev. *21*, 1720–1725.
5. Berger, D., and Altmann, T. (2000). A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in *Arabidopsis thaliana*. Genes Dev. *14*, 1119–1131.
6. Von Groll, U., Berger, D., and Altmann, T. (2002). The subtilisin-like serine protease SDD1 mediates cell-to-cell signaling during Arabidopsis stomatal development. Plant Cell *14*, 1527–1539.

7. Yang, M., and Sack, F.D. (1995). The too many mouths and four lips mutations affect stomatal production in Arabidopsis. Plant Cell *7*, 2227–2239.
8. Nadeau, J.A., and Sack, F.D. (2002). Control of stomatal distribution on the *Arabidopsis* leaf surface. Science *296*, 1697–1700.
9. Shpak, E.D., McAbee, J.M., Pillitteri, L.J., and Torii, K.U. (2005). Stomatal patterning and differentiation by synergistic interactions of receptor kinases. Science *309*, 290–293.
10. Bergmann, D.C., Lukowitz, W., and Somerville, C.R. (2004). Stomatal development and pattern controlled by a MAPKK kinase. Science *304*, 1494–1497.
11. Wang, H., Ngwenyama, N., Liu, Y., Walker, J.C., and Zhang, S. (2007). Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in Arabidopsis. Plant Cell *19*, 63–73.
12. Ohashi-Ito, K., and Bergmann, D. (2006). *Arabidopsis* FAMA controls the final proliferation/differentiation switch during stomatal development. Plant Cell *18*, 2493–2505.
13. MacAlister, C.A., Ohashi-Ito, K., and Bergmann, D.C. (2006). Transcription factor control of asymmetric cell divisions that establish the stomatal lineage. Nature *445*, 537–540.
14. Pillitteri, L.J., Sloan, B.D., Bogenschutz, N.L., and Torii, K.U. (2006). Termination of asymmetric cell division and differentiation of stomata. Nature *445*, 501–505.
15. Kutter, C., Schöb, H., Stadler, M., Meins, F., Jr., and Si-Ammour, A. (2007). MicroRNA-mediated regulation of stomatal development in Arabidopsis. Plant Cell *19*, 2417–2429.
16. Lampard, G.R., MacAlister, C., and Bergmann, D.C. (2008). Arabidopsis stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. Science *322*, 1113–1116.
17. 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.
18. Matsubayashi, Y., and Sakagami, Y. (2006). Peptide hormones in plants. Annu. Rev. Plant Biol. *57*, 649–674.
19. Fletcher, J.C., Brand, U., Running, M.P., Simon, R., and Meyerowitz, E.M. (1999). Signaling of cell fate decisions by *CLAVATA3* in *Arabidopsis* shoot meristems. Science *283*, 1911–1914.
20. Gray, J.E., Casson, S., and Hunt, L. (2008). Intercellular peptide signals regulate plant meristematic cell fate decisions. Sci Signal *1*, pe53.
21. Alonso, J.M., Stepanova, A.N., Leisse, T.J., Kim, C.J., Chen, H., Shinn, P., Stevenson, D.K., Zimmerman, J., Barajas, P., and Cheuk, R. (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science *301*, 653–657.
22. Rosso, M.G., Lim, Y., Strizhovm, N., Reiss, B., Dekker, K., and Weisshaar, B. (2003). An Arabidopsis thaliana T-DNA mutagenized population (GABI-Kat) for flanking sequence tag-based reverse genetics. Plant Mol. Biol. *53*, 247–259.
23. Karimi, M., Inze, D., and Depicker, A. (2002). GATEWAY vectors for Agrobacterium-mediated plant transformation. Trends Plant Sci. *7*, 193–195.
24. Rohila, J.S., Chen, M., Cerny, R., and Fromm, M.E. (2004). Improved tandem affinity purification tag and methods for isolation of protein heterocomplexes from plants. Plant J. *38*, 172–181.
25. Clough, S.J., and Bent, A.F. (1998). Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. *16*, 735–743.