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Article Addendum

A Shout-Out to Stomatal Development

How the bHLH Proteins SPEECHLESS, MUTE and FAMA Regulate Cell Division and Cell Fate

Gregory R. Lampard

Dominique C. Bergmann

Department of Biological Sciences; Stanford University; Stanford, California USA

*Correspondence to: Gregory R. Lampard; Department of Biological Sciences; Stanford University; Stanford, California 94305-5020 USA; Tel.: 650.736.0983; Fax: 650.725.8309; Email: glampard@stanford.edu/Dominique C. Bergmann; Department of Biological Sciences; Stanford University; Stanford, California 94305-5020 USA; Tel.: 650.736.0983; Fax: 650.725.8309; Email: dbergmann@stanford.ed

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Addendum to:

Transcription Factor Control of Asymmetric Cell Divisions that Establish the Stomatal Lineage

MacAlister CA, Ohashi-Ito K, Bergmann DC

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and

Arabidopsis FAMA Controls the Final Proliferation/Differentiation Switch During Stomatal Development

Ohashi-Ito K, Bergmann DC

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ABSTRACT

Arabidopsis guard cell development requires a three step series of asymmetric and symmetric divisions followed by terminal differentiation. We have recently identified three paralogous bHLH transcription factors, SPEECHLESS, MUTE and FAMA, that each function as a master regulator of a specific stage of stomatal development. These findings provide the expected counterbalance to the previously described negative regulatory signaling network and raise intriguing new questions about relationships among the regulators that ultimately enable proper stomatal development and pattern.

INTRODUCTION

Guard cells (GCs) control the aperture of the major conduit for gas exchange by plants. Both the activity and the production of GCs respond to fluctuating biotic and abiotic conditions. While GC function has been extensively studied (reviewed in refs. 1 and 2), less is known about signaling mechanisms that drive stomatal specification and differentiation. Aspects of this developmental pathway make it an ideal model to study cellular development and cell fate. While the signaling mechanisms that ultimately control stomatal fate are representative of those broadly used in plant development, several of the molecules appear to be specific to stomata, providing a closed system in which gene and protein function can be manipulated with minimal cross-talk with other aspects of plant development. In addition, elucidation of the mechanisms directing stomatal cell fate will illuminate the ability of plant cells to coordinate physiological and developmental processes within a single cell and how these processes can be coordinated between generations of mother, daughter and sister cells.

CONTROL OF STOMATAL DEVELOPMENT IN ARABIDOPSIS

Arabidopsis GC development occurs via a stepwise series of asymmetric and symmetric cell divisions (Fig. 1). The number and placement of these divisions is dependent on the activities of classical signal-transduction proteins including receptor-like proteins and a mitogen activated protein kinase (MAPK) signaling module,[3-7] all of which negatively regulate stomatal development.

Transcription factors encoding positive regulators of stomatal formation and differentiation have also been identified. The first of these were a pair of closely related MYBs that, although not absolutely required for the production of stomata, promote the timely transition from division to differentiation at the last step in the formation of GCs.[8] More recently three closely related bHLH transcription factors were discovered to promote each of the specific transition steps in a three-step GC development model[9-11] (Fig. 1). SPEECHLESS (SPCH) protein expression is restricted to undifferentiated epidermal cells, and the leaf epidermis of *spch* loss-of-function plants contains only pavement cells and trichomes, suggesting that *SPCH* controls asymmetric entry divisions.[9] *MUTE* functions in promoting the transition from meristemoid to guard mother cell (GMC) as demonstrated by the phenotype of *mute* loss-of-function plants that display additional rounds of asymmetric divisions resulting in the production of a meristemoid surrounded by a rosette pattern of incompletely differentiated cells.[10] Consistent with this, overexpression of *MUTE* results in the conversion of all epidermal cells to mature GCs.[9,10] *FAMA*, originally identified by a transcriptional profiling experiment as differentially regulated upon activation or elimination of the MAPKKK YODA, controls the final transition from GMC to mature GCs.[11] *fama* loss-of-function plants also fail to produce mature stomata, with

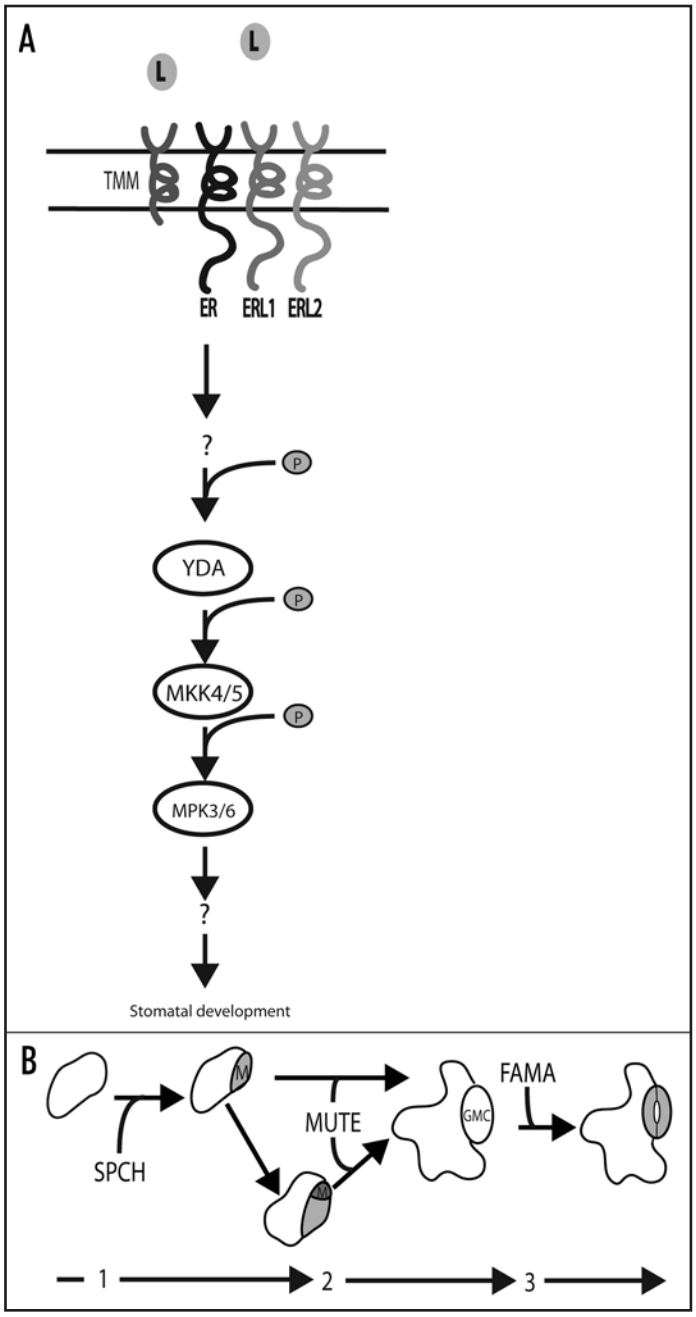

Figure 1. (A) Intracellular signaling module. Unidentified ligands interact with multiple receptors (ER, ERL1, ERL2 and TMM) to activate the YDA-MKK4/5-MPK3/6 MAPK module which regulates entry into the stomatal lineage. (B) Positive regulators of stomatal development. Guard cells are formed by three stages of asymmetric and symmetric cell divisions (1-3). Passage to each subsequent stage is promoted by SPCH (1; asymmetric entry division), MUTE (2; meristemoid (M) to guard mother cell (GMC)) and FAMA (3; GMC to guard cells).

mutant plants developing caterpillar-like tumors on the epidermal surface that arise from continual division of GMCs without terminal differentiation.[11] In further support of this model, overexpression of *FAMA* converts all epidermal cells to unpaired GC-like cells.[11]

Identifying the positive regulators was a breakthrough for our understanding of how stomata are constructed. The fact that three paralogous bHLHs function as master regulators of each of the stages in stomatal development poses several interrelated questions about regulatory networks and gene evolution. How do these transcription factors function to drive stomatal development? Which genes are controlled by each respective transcription factor, and are the later expressed bHLHs targets of the earlier ones? How is the specific spatial and temporal pattern of each bHLH protein regulated? Does the resemblance between control of plant and animal cell fate through the adoption of multiple bHLHs indicate an underlying conservation of developmental logic? In this addendum we will explore some of these questions raised by our recent publications on stomatal development.

WHAT IS THE RELATIONSHIP BETWEEN EACH OF THE BHLHS AND HOW ARE THEY REGULATED?

It is apparent that the three bHLH transcription factors, SPCH, MUTE and FAMA each promote a specific step in the stomatal development pathway.[9-11] However, what has not been extensively explored is the relationship among these bHLHs. One plausible regulatory scenario is that expression of each gene is controlled directly by the previously acting one (i.e., SPCH activity directly results in transcriptional activation of *MUTE*). Conversely, one might postulate an antagonistic mechanism in which expression/action of the earlier transcription factor is down-regulated directly by the activity of the next bHLH. Finally, the three transcription factors may function independently. In this third model, the proteins function generally in promoting the passage to the next developmental stage and this triggers induction of the sequential transcription factor.

Expression of FAMA is dependent on functional MUTE[10] and SPCH.[9] Therefore, formally, each of the earlier acting transcription factor(s) is required for expression of the last. However, given that the translational reporters for SPCH, MUTE and FAMA show peak expression in non-overlapping cell types, it appears that each successive bHLH neither directly activates nor represses the next. Whether the later proteins have antagonistic effects on the cellular processes promoted by the former might be determined by identifying the common targets of each transcription factor and subsequently analyzing the effect each has on the expression those common genes. That FAMA overexpression in a *spch* loss-of-function background results in the formation of unpaired GCs[9] suggests that on some levels the processes controlled by SPCH, MUTE and FAMA are independent.

Understanding how each of the transcription factors actually function at a biochemical level may clarify the nature of the regulatory relationships among the stomatal bHLHs; do they function as transcriptional activators, repressors, or both? Do they function by binding to DNA directly and/or by interacting with other proteins? The answers to these questions likely involve aspects of both scenarios. For example, FAMA appears to function as a transcriptional activator and contains a conserved DNA binding motif, yet, elimination of the DNA binding ability by converting the conserved -HER- residues in the DNA binding domain to non-charged amino acids ($FAMA_{PGG}$) does not create, as was expected, a dominant negative version of FAMA.[11] Rather, expression of $FAMA_{PGG}$ in a wild type background converts all epidermal cells into mature, paired GCs.[11] This phenotype is identical to that caused by overexpression of wild type *MUTE*[9,10] and may be indicative that FAMA functions both by directly binding DNA to activate transcription and by modulating the activity of other proteins through physical interactions. FAMA was shown to interact with bHLH071 and bHLH093 in a yeast two hybrid assay and an

in planta split-GFP experiment.[11] However, the functional relevance of these interactions is not clear; neither loss-of-function mutations in, nor overexpression of, bHLH071 and bHLH093 produce strong phenotypes.[11] Identifying partners relevant for stomatal development may require coimmunoprecipitation assays in the appropriately staged tissues. These experiments, when coupled with global target analyses such as ChIP assays for each of SPCH, MUTE and FAMA will provide starting points to characterize the regulatory networks surrounding each step of stomatal development.

CONCLUSIONS

While many of the proteins involved in stomatal development have been identified, much remains to be learned in regards to the control of stomatal fate. Understanding this relatively straight-forward "three-step" cell fate pathway may provide both an understanding of terminal differentiation of plant cells and the ability to have greater control over stomatal development and function. This could ultimately enable the production of highly stress-tolerant plants.

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