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Plasticity in stomatal development

What role does MAPK signaling play?

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Stomata are specialized pores found on the epidermal surface of many aerial tissues of plants, where they function to regulate the exchange of gases such as carbon dioxide and water vapor between the plant and its environment. This makes stomatal complexes essential for the survival of the plant; a complete loss of stomata is lethal. On a global level, stomatal regulation of gas exchange makes stomata critical regulators of carbon and water cycles, while on an organismal level, stomatal development is flexible in that the ultimate distribution of stomata can be controlled by environmental stimuli.[1] While several environmental factors capable of influencing stomatal development have been identified, the molecular mechanisms mediating this flexibility have remained elusive. Recent studies suggest that this plasticity involves an expanding collection of mitogen activated protein kinase (MAPK) signaling components and putative upstream extracellular ligands.[2,3] Furthermore, it appears that stomatal development and distribution may not be the result of a simple "on/off" switch regulating lineage entry. Rather, stomatal precursors in Arabidopsis can be influenced at multiple points in the well-characterized stomatal development pathway by modulation of a core MAPK signaling module.[3]

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Stomatal Development in Arabidopsis

Stomatal development in Arabidopsis follows a series of symmetric and asymmetric cell division and differentiation steps (**Fig. 1**).[4] A number of both positive and negative regulators of stomatal development have been identified. Negative regulators include components of a MAPK signaling module initially identified as consisting of the MAPKKK YDA, the MAPKKs MKK4/5, and the MAPKs MPK3 and MPK6 (**Fig. 1**).[5,6] In addition, two additional stress- and phytohormone-associated MAPKKs, MKK7 and MKK9, are now known to be capable of inhibiting stomatal development (**Fig. 1**).[3] Though biochemical data are lacking, genetic evidence places the MAPK module downstream of the leucine rich repeat (LRR) receptor-like protein TOO MANY MOUTHS (TMM) and a family of LRR receptor-like protein kinases, ERECTA, ERL1 and ERL2 (**Fig. 1**). At least two negatively acting putative ligands, EPF1 and EPF2, appear to function upstream of these receptors.[7-10] It is unlikely that all stomatal-regulatory signaling feeds through the MAPK module, as the putative protease SDD1 negatively regulates stomatal development independently of the aforementioned signaling molecules.[2,11]

Entry into and progression through the stomatal development pathway are promoted by three bHLH transcription factors, SPCH, MUTE and FAMA, each of which regulates a specific transition (**Fig. 1**).[4] Additional bHLH transcription factors, SCRM1 and SCRM2, also promote stomatal development and appear to function in each transition stage through direct interactions with SPCH, MUTE or FAMA.[12]

Initial reports placed the MAPK module directly upstream of the entry divisions that initiate stomatal development.[5,6] Specifically, MPK3 and MPK6 control entry into the

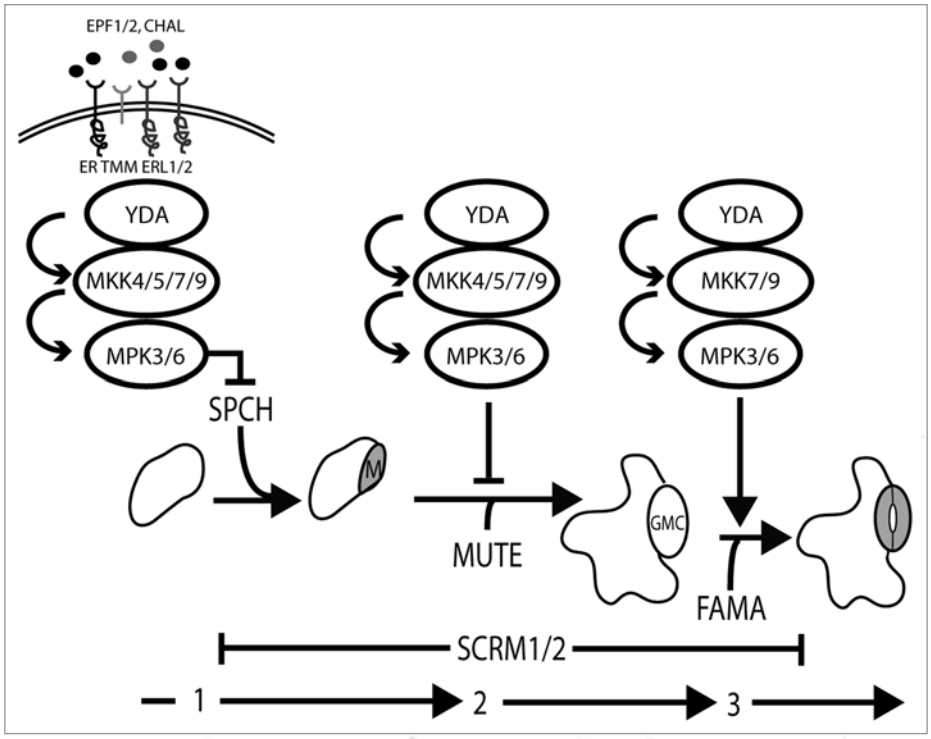

Figure 1. Current model of stomatal development. The related bHLH transcription factors SPCH, MUTE and FAMA promote asymmetric entry divisions (1), differentiation of meristemoids (M) into guard mother cells (GMC; 2) and terminal guard mother cell to guard cell division and differentiation steps (3) respectively. Each of these stages is also influenced by the actions of additional bHLH transcription factors SCRM1 and SCRM2, likely through interactions with each of SPCH, MUTE and FAMA. Entry into the stomatal lineage is negatively regulated by the YDA-MKK4/5/7/9-MPK3/6 MAPK signaling module. Activation of this MAPK module appears to be controlled by the ER-family of receptors (ER, ERL1, ERL2) and Too Many Mouths (TMM), which themselves are regulated by interactions with the putative extracellular ligands EPF1/2 and CHAL. Activity of this core MAPK module can also block the M to GMC transition, while a partially overlapping MAPK module (MKK4 nor MKK5 are implicated in this process) promotes terminal guard cell development. Upstream activators of the MAPK module during stages beyond entry divisions have yet to be identified.

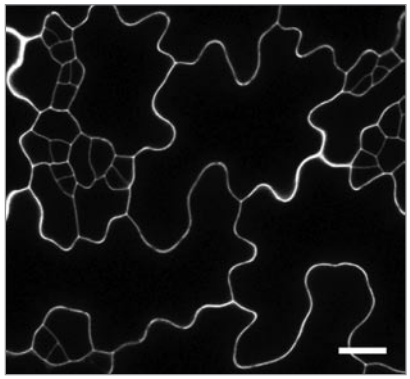

Figure 2. 7-day old adaxial cotyledon of a transgenic Arabidopsis plant expressing a constitutively active form of MKK9 in meristemoids using the *MUTE* promoter sequences. Activation of MKK9 in meristemoids results in arrested stomatal development and a build-up of meristemoid like cells. Activation of any of, YDA, MKK4, MKK5 or MKK7 in meristemoids produces a similar phenotypic outcome. Scale bar = 50 μM.

stomatal lineage by directly controlling the phosphorylation status of SPCH.[5] SPCH can be phosphorylated by both MPK3 and MPK6 and hyper-phosphorylation correlates with diminished SPCH activity, yet a specific phosphorylation site, S193, is essential for SPCH to promote stomatal development.[5] Thus, SPCH activity may be subject to both positive and negative regulation by the MAPK module at this level.

Recent work from Lampard et al. (2009)[3] not only identifies new MAPKKs (MKK7 and MKK9) capable of blocking stomatal lineage entry, but also suggests a potential role for MAPK activity in regulating subsequent pathway stages. Specifically, activation of YDA or any putative downstream MAPKK (MKK4/5/7/9) in meristemoids inhibits lineage progression, creating an epidermis containing apparently normal pavement cells, small meristemoid-like cells, and no mature stomata (**Fig. 2**). Therefore, in addition to negatively regulating SPCH, the MAPK module can inhibit developmental transitions normally promoted by MUTE. It should be noted that MAPKK inhibition of stomatal development is not a general phenomenon for all MAPKKs; activation of MKK1, MKK2 or MKK6 in meristemoid mother cells, meristemoids or guard mother cells does not affect stomatal development.[3]

These findings raise three salient questions: (1) Why are so many MAPKKs capable of perturbing stomatal development? (2) Why is there control over stomatal development at multiple stages? (3) What are the targets of the MAPK modules, particularly in meristemoids, where MUTE is not an in vitro substrate of MPK3 or MPK6?

Plasticity in Stomatal Development

Stomatal development in Arabidopsis and other plants is flexible. One example

is tissue-specific plasticity, evident in the marked differences in stomatal density between tissue types and even opposite surfaces of leaves.[13] Another example is plasticity in response to environmental cues, with inputs including heat, light and carbon dioxide levels known to dramatic alter both stomatal density and index.[1,14] That the MAPK signaling network including MKK4/5/7/9 and MPK3/6 is involved in stomatal development is thus not surprising. Each of these kinases has been thoroughly characterized and each controls various responses of the plant to abiotic and biotic stresses (reviewed in ref. 15). Given that stresses of these types trigger large-scale downregulation of developmentally associated gene expression, one can envision a model in which this MAPK module plays a central role controlling plasticity in stomatal development (**Fig. 3**). Stresses, be they biotic or abiotic, would trigger activation of the MAPK module in multiple cell types, including but not limited to meristemoid mother cells and meristemoids. Amongst the responses evoked by MAPK activity would be inhibited stomatal development. It will be intriguing to determine how flexible this model is; for example, is this inhibition reversible? In this case, stomatal development might be "paused" while the plant mounts an appropriate stress-response and resumed once that stress is alleviated.

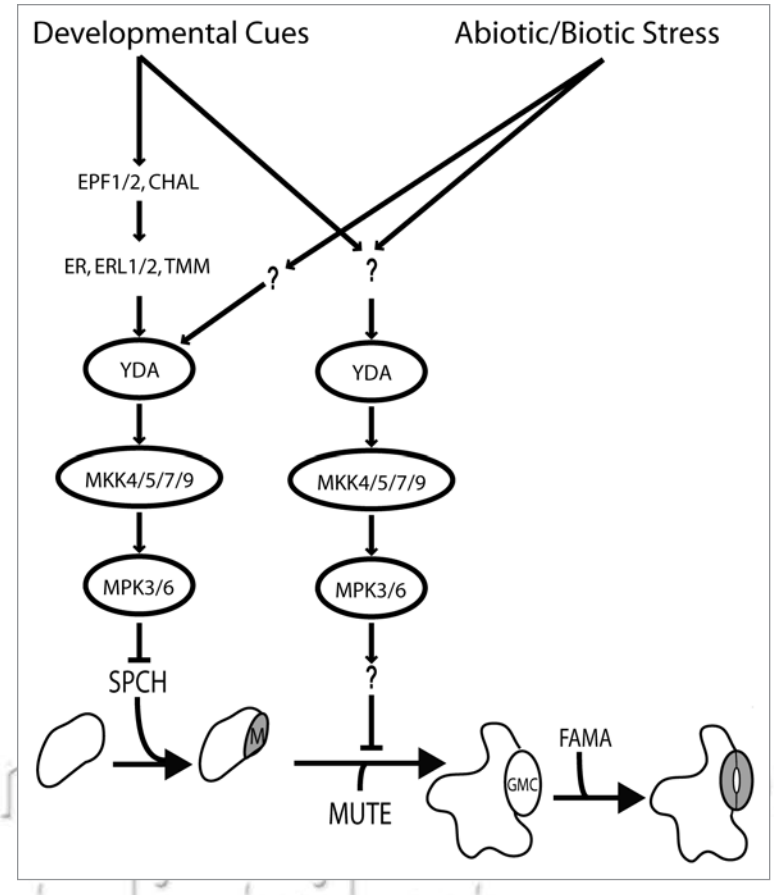

Figure 3. Hypothetical model of the integration of developmental and abiotic/biotic stress signals through a common MAPK module to control stomatal development. A common MAPK module negatively regulates both entry into and early progression (M to GMC transition) through the stomatal lineage. Each of the MAPKKs and MAPKs has been implicated in controlling stress-responses, suggesting that cross-talk between abiotic/biotic stress responses and developmental cues occurs at this at this level. Developmental signals likely control the expression patterns of the EPF family members, EPF1/2 and CHAL. Subsequent interaction of these ligands with the ER-family members and TMM would ultimately control activity and fine tuning of entry into the stomatal lineage by initiating signaling through the MAPK module at this stage. Possible functions of the EPF or ER family members in controlling later stages of stomatal development or stress-induced alteration in stomatal patterning have not been identified.

In dicots such as Arabidopsis, where stomatal development occurs concurrently throughout a developing leaf, it will be interesting to determine whether stress-induced effects are local, systemic or both. Currently, it is known that a light-induced signal can be detected in mature tissues and this signal leads to altered stomatal densities in future leaves.[1] This new model would suggest that in the case of acute stresses, local development could be temporarily arrested and resumed following alleviation of the stress. Testing of this model would be greatly simplified by developing cell-type specific inducible MAPK activation systems: This would allow direct examination of phenotypic outcomes following artificial activation and subsequent deactivation in individual cell types of the stomatal lineage. Tests of this nature could provide broader insight into how plants alter developmental regimes in stressful conditions. When coupled with a greater characterization of specific events controlled by each of the MAPKKs involved, these types of experiments may also ultimately reveal why numerous MAPKKs are capable of influencing stomatal development.

If indeed, plants are capable of placing developmental processes such as stomatal development on hold during stress responses, the data will fit with current data reflecting the ability of numerous MAPKKs to inhibit stomatal development at the level of the meristemoid. The ability to pause development at multiple points might allow more efficient utilization of resources for mounting effective stress-responses.

In addition to stress-induced plasticity in stomatal development, there is also regional plasticity in stomatal development patterns. The above model suggests why a common MAPK could control the stress-induced plasticity, but does not explain how the same module might be involved in regional plasticity. Genetic evidence places the MAPK module downstream of the ER-family of LRR-receptors and the putative ligands EPF1 and EPF2.[2,7,8] The number of putative ligands acting upstream of the MAPK module is increasing: the EPF gene family member *CHAL* encodes a putative ligand that negatively regulates stomatal development.[2] It is hypothesized that, through interactions with ER family

members and tissue-specific differences in expression, CHAL inhibits stomatal development in specific tissue types and thus contributes to regional plasticity of stomatal development. Using a common MAPK control module that includes numerous MAPKKK, MAPKK and MAPK gene family members agrees with the model that MAPK signaling in plants has evolved to enable the convergence of numerous upstream stimuli into a core response element that subsequently initiates divergent cellular responses.

What are the Other Key Regulators of Stomatal Development?

The current model of stomatal development is incomplete. Sufficient data support the notion that there are (1) unidentified effector proteins lying downstream of the MAPK module, particularly in meristemoids[3] and (2) yet-to-be-characterized ligands controlling stomatal development.[2] Identifying the targets of MPK3 and MPK6 in meristemoids will refine our understanding of MAPK control over meristemoid behavior and may more broadly clarify how this module functions in multiple stomatal lineage cell types. For example, in silico data suggest that the more widely expressed bHLH genes *SCRM1* and *SCRM2* encode putative MAPK substrates. If this is the case, the extent to which this phosphorylation impacts stomatal development in multiple cell types will need to be examined.

Beyond the MAPK substrates, untangling the complex interactions between the EPF family of ligands and the ER family of receptors will no doubt add insight into current models on how plants control stomatal development in different tissue types. The extent to which, if at all, these ligands and receptors overlap with upstream transducers of environmental inputs will shed further light onto the extent to which MAPK signaling can be viewed as a master integrator in plasticity of stomatal development.

References

1. Casson S, Gray J. Influence of environmental factors on stomatal development. New Phytol 2008; 178:9-23.
2. Abrash EB, Bergmann DC. Regional specification of stomatal production by the putative ligand CHALLAH. Development 2010; 137:447-55.
3. Lampard GR, Lukowitz W, Ellis BE, Bergmann DC. Novel and expanded roles for MAPK signaling in Arabidopsis stomatal cell fate revealed by cell type-specific manipulations. Plant Cell 2009; 21:3506-17.
4. Bergmann DC, Sack FD. Stomatal development. Annu Rev Plant Biol 2007; 58:163-81.
5. Lampard GR, MacAlister CA, Bergmann DC. Arabidopsis stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. Science 2008; 322:1113-6.
6. Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S. Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in Arabidopsis. Plant Cell 2007; 19:63-73.
7. Hara K, Kajita R, Torii KU, Bergmann DC, Kakimoto T. The secretory peptide gene EPF1 enforces the stomatal one-cell-spacing rule. Genes Dev 2007; 21:1720-5.
8. Hunt L, Gray JE. The signaling peptide EPF2 controls asymmetric cell divisions during stomatal development. Curr Biol 2009; 19:864-9.
9. Serna L. Cell fate transitions during stomatal development. Bioessays 2009; 31:865-73.
10. Shpak ED, McAbee JM, Pillitteri LJ, Torii KU. Stomatal patterning and differentiation by synergistic interactions of receptor kinases. Science 2005; 309:290-3.
11. Von Groll U, Berger D, Altmann T. The subtilisin-like serine protease SDD1 mediates cell-to-cell signaling during arabidopsis stomatal development. Plant Cell 2002; 14:1527-39.
12. Kanaoka MM, Pillitteri LJ, Fujii H, Yoshida Y, Bogenschutz NL, Takabayashi J, et al. SCREAM/ICE1 and SCREAM2 specify three cell-state transitional steps leading to Arabidopsis stomatal differentiation. Plant Cell 2008; 20:1775-85.
13. Geisler M, Yang M, Sack FD. Divergent regulation of stomatal initiation and patterning in organ and suborgan regions of the Arabidopsis mutants too many mouths and four lips. Planta 1998; 205:522-30.
14. Casson SA, Franklin KA, Gray JE, Grierson CS, Whitelam GC, Hetherington AM. Phytochrome B and PIF4 regulate stomatal development in response to light quantity. Curr Biol 2009; 19:229-34.
15. Colcombet J, Hirt H. Arabidopsis MAPKs: A complex signalling network involved in multiple biological processes. Biochem J 2008; 413:217-26.