Article:

Chater, C. and Gray, J.E. (2015) Stomatal Closure: The Old Guard Takes Up the SLAC. Current Biology, 25 (7). R271 - R273. ISSN 0960-9822

https://doi.org/10.1016/j.cub.2015.01.032

The Old Guard takes up the SLAC: the evolution of active stomatal closure

Caspar Chater; Julie E. Gray

Department of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield, S10 2TN, UK

Summary

Flowering plant stomata close via passive dehydration or by active pumping of anions through SLAC, a phospho-activated membrane channel. The demonstration that moss likely utilise this same mechanism supports an early origin for SLAC-mediated active stomatal control.

Stomata are microscopic pores on above-ground plant surfaces, formed by guard cells. CO_2, for photosynthesis, diffuses in through stomata whilst water is transpired out. Active control of stomatal closure to limit water loss was arguably one of the most critical steps in land plant dominance of the terrestrial environment, but the origins of this significant evolutionary development remain controversial. Flowering plants, the most recently diverged and most diverse group of land plants, have highly complex stomatal control systems to open and close their stomata in response to factors such as light, CO_2 concentration, and drought. In this way, they optimise carbon gain and minimise water loss under constantly fluctuating environmental conditions. Stomata first appear in the fossil record in bryophyte-like early land plants but when did this ability to fine-tune transpiration first arise? A new study by Lind *et al.* [1] clearly demonstrates that the last common ancestor of mosses and vascular plants possessed an OST1 kinase-activated SLAC ion channel, which is a crucial component of active stomatal closure.

ABA is a drought response hormone which regulates gene expression and active stomatal closure in flowering plants. Significant progress has been made in our understanding of the core ABA signalling pathway [2]. ABA activates PYR receptors, which inhibit the PP2C phosphatases, thereby transducing a signal via SnRK2 kinases to activate downstream processes (Fig. 1A). This pathway evolved in an ancestor of all extant land plants to regulate expression of genes conferring desiccation and drought tolerance [1, 3]. Homologs of ABA signalling components are found in liverworts, mosses and lycophytes (the extant bryophytes) [4-8] and these components have been shown to confer drought tolerance in liverwort and moss gametophytes [3, 9-12]. There is consensus agreement that the ABA signalling pathway’s complexity increased in the ancestor of bryophytes to confer greater control over desiccation tolerance [1, 3, 10, 12-14].

The ABA signalling pathway’s role in regulating the stomata of flowering plants requires a specialised SnRK2 called OST1. As well as phosphorylating transcription factors, in guard cells OST1 also activates an anion channel SLAC1, resulting in a reduction in turgor and active stomatal closure (Fig. 1A). This guard cell specific innovation in the pathway led to the fine spatial and temporal control of transpiration observed in flowering plants but the physiological and ecological relevance of the guard cell ABA signalling pathway in early divergent land plant lineages remains controversial [15, 16]. Several studies have shown that the stomata of some ferns and early divergent seed plants respond minimally to ABA suggesting that in these species stomatal closure may be a passive hydraulic response, rather than actively mediated by ABA [17-19]. This has resulted in the hypothesis that active SLAC-induced stomatal closure arose after the bryophytes in the ancestor of flowering plants [3, 18, 19].

Lind *et al.* studied the interactions of homologs of SLAC1 and OST1 from an early divergent charophyte alga, an astomate liverwort, a stomatous moss (*Physcomitrella patens*, Fig. 1B and C) and those of the model flowering plant Arabidopsis, to examine the origins of this guard cell-specific response [1]. They demonstrate that moss and flowering plant OST1 proteins can activate the moss SLAC channel and thereby potentially transduce an ABA signal in moss stomata [1]. OST1-like proteins from all of the species tested activated ABA-inducible genes when expressed in Arabidopsis protoplasts in the presence of ABA. This is in line with the evidence that transcriptional regulation by ABA is an ancient response pathway predating the origins of land plants. Similarly, all OST1-like proteins tested, including those of the astomate algal and liverwort species, activated Arabidopsis SLAC1 when expressed in Xenopus oocytes. In each case, this activation could be blocked by expression of a PP2C phosphatase. However, only the OST1 proteins from Arabidopsis or the moss Physcomitrella (the two species tested that possess guard cells) were able activate Physcomitrella SLAC1. This suggested that there is something distinct about the OST1-mediated activation of SLAC1 in moss.

Further experiments in Xenopus oocytes showed that the moss OST1 can physically interact with moss or Arabidopsis SLAC1, and that this leads to anion channel activation [1]. Two serine residues in the N-terminal regulatory region of Arabidopsis SLAC1 that are known to be targets for OST1 phosphorylation are conserved between Arabidopsis and Physcomitrella, but are not present in the astomate algal and liverwort anion channels. Together with an extended C-terminal region, the acquisition of these putative phosphorylation sites appears to be the important distinction for the activation of Physcomitrella SLAC1 and hence in the evolution of stomatal function.

There is further evidence to support the hypothesis that the SnRK2s were recruited early in land plant evolution to mediate active guard cell responses. The stomata of moss and lycophyte species appear structurally similar to those of flowering plants (Fig 1C) and they close in response to ABA. Their OST1 kinases mediate stomatal closure when expressed in Arabidopsis guard cells [7, 8, 20], and ABA-inducible stomatal closure is compromised in a moss mutant lacking Physcomitrella OST1-1 [7]. The work of Lind *et al.* provides molecular confirmation of moss SLAC and OST1 interaction, which is strong evidence for the co-option of the ABA-signalling pathway for active stomatal aperture control in bryophyte-like early land plants [6, 7, 20]. These latest data suggest that active stomatal closure was acquired very early in the history of stomata, in a period during which plants conquered the land masses of the Earth.

References

1. Lind, C., Dreyer I., López-Sanjurjo, E. J., von Meyer, K., Ishizaki, K., Kohchi, T., Lang, D., Zhao, Y., Kreuzer, I., Al-Rasheid, K.A.S., Ronne, H., Reski, R., Zhu, J.-K., Geiger, D., and Hedrich, R. (2015). Stomatal guard cells co-opted an ancient ABA-dependent desiccation survival system to regulate stomatal closure. Curr. Biol.
2. Cutler, S.R., Rodriguez, P.L., Finkelstein, R.R., and Abrams, S.R. (2010). Abscisic Acid: Emergence of a Core Signaling Network. Ann. Rev. Plant Biol. *61*, 651-679.
3. Komatsu, K., Suzuki, N., Kuwamura, M., Nishikawa, Y., Nakatani, M., Ohtawa, H., Takezawa, D., Seki, M., Tanaka, M., Taji, T., *et al.* (2013). Group A PP2Cs evolved in land plants as key regulators of intrinsic desiccation tolerance. Nat. Commun. *4*.
4. Hauser, F., Waadt, R., and Schroeder, J.I. (2011). Evolution of Abscisic Acid Synthesis and Signaling Mechanisms. Curr. Biol. *21*, R346-R355.
5. Banks, J.A., Nishiyama, T., Hasebe, M., Bowman, J.L., Gribskov, M., dePamphilis, C., Albert, V.A., Aono, N., Aoyama, T., Ambrose, B.A., *et al.* (2011). The Selaginella Genome Identifies Genetic Changes Associated with the Evolution of Vascular Plants. Science *332*, 960-963.
6. O'Donoghue, M.-T., Chater, C., Wallace, S., Gray, J.E., Beerling, D.J., and Fleming, A. (2013). Genome-wide transcriptomic analysis of the sporophyte of the moss *Physcomitrella patens*. J. Ex. Bot. 64(12), 3567-81
7. Chater, C., Kamisugi, Y., Movahedi, M., Fleming, A., Cuming, A.C., Gray, J.E., and Beerling, D.J. (2011). Regulatory Mechanism Controlling Stomatal Behavior Conserved across 400 Million Years of Land Plant Evolution. Curr. Biol. *21*, 1025-1029.
8. Ruszala, E., Beerling, D.J., Franks, P.J., Chater, C., Casson, S.A., Gray, J.E., and Hetherington, A.M. (2011). Land Plants Acquired Active Stomatal Control Early in Their Evolutionary History. Curr. Biol. *21*, 1030-1035.
9. Cuming, A.C., Cho, S.H., Kamisugi, Y., Graham, H., and Quatrano, R.S. (2007). Microarray analysis of transcriptional responses to abscisic acid and osmotic, salt, and drought stress in the moss, *Physcomitrella patens*. New Phyt. *176*, 275-287.
10. Komatsu, K., Nishikawa, Y., Ohtsuka, T., Taji, T., Quatrano, R., Tanaka, S., and Sakata, Y. (2009). Functional analyses of the ABI1-related protein phosphatase type 2C reveal evolutionarily conserved regulation of abscisic acid signaling between Arabidopsis and the moss *Physcomitrella patens*. Plant Mol. Biol. *70*, 327-340.
11. Bhyan, S.B., Minami, A., Kaneko, Y., Suzuki, S., Arakawa, K., Sakata, Y., and Takezawa, D. (2012). Cold acclimation in the moss Physcomitrella patens involves abscisic acid-dependent signaling. J. Plant Phys. *169*, 137-145.
12. Takezawa, D., Watanabe, N., Ghosh, T.K., Saruhashi, M., Suzuki, A., Ishiyama, K., Somemiya, S., Kobayashi, M., and Sakata, Y. (2014). Epoxycarotenoid-mediated synthesis of abscisic acid in *Physcomitrella patens* implicating conserved mechanisms for acclimation to hyperosmosis in embryophytes. New Phyt. DOI: 10.1111/nph.13231
13. Takezawa, D., Komatsu, K., and Sakata, Y. (2011). ABA in bryophytes: how a universal growth regulator in life became a plant hormone? J. Plant Res. *124*, 437-453.

14. Wang, C., Liu, Y., Li, S-S and Han, G-Z. (2015) Insights into the origin and evolution of plant hormone signaling machinery. Plant Phys, *in press*.

15. Ligrone, R., Duckett, J.G., and Renzaglia, K.S. (2012). Major transitions in the evolution of early land plants: a bryological perspective. Ann. Bot. *109*, 851-871.
16. Haig, D. (2013). Filial mistletoes: the functional morphology of moss sporophytes. Ann. Bot. *111*, 337-345.
17. McAdam, S.A.M., and Brodribb, T.J. (2014). Separating active and passive influences on stomatal control of transpiration. Plant Phys. *164*, 1578-1586.
18. Brodribb, T.J., and McAdam, S.A.M. (2010). Passive origins of stomatal control in vascular plants. Science *331*, 582-585.
19. McAdam, S.A.M., and Brodribb, T.J. (2012). Fern and lycophyte guard cells do not respond to endogenous abscisic acid. Plant Cell *24*, 1510-1521.
20. Chater, C., Gray, J.E., and Beerling, D.J. (2013). Early evolutionary acquisition of stomatal control and development gene signalling networks. Curr. Op. Plant Biol. *16*, 638-646.

Figure 1.

The evolution of guard cell-specific ABA signalling and active stomatal closure. A. Schematic of the core ancestral ABA signalling pathway regulating transcription (red) and the derived guard cell specific OST1-SLAC1 mechanism for stomatal closure via plasma membrane anion efflux (green). B. z-stacked LSM image of a Physcomitrella spore capsule attached to its parent gametophore. Propidium iodide stain outlines the cells and highlights the ring of stomata around the base of the capsule (green). Chloroplasts autofluoresce red. Scale = 25 µm. C. Detail of a single Physcomitrella stoma from B, revealing its structural similarity to stomata of the later diverging flowering plants. Scale bar = 10 µm. Confocal images C. Chater, S. A. Rolfe.