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

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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


**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.