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Review

The role of brassinosteroids and abscisic acid in stomatal development

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ABSTRACT

Gas exchange with the atmosphere is regulated through the stomata. This process relies on both the degree and duration of stomatal opening, and the number and patterning of these structures in the plant surface. Recent work has revealed that brassinosteroids and abscisic acid (ABA), which control stomatal opening, also repress stomatal development in cotyledons and leaves of at least some plants. It is speculated that, in Arabidopsis, these phytohormones control the same steps of this developmental process, most probably, through the regulation of the same mitogen-activated protein (MAP) kinase module. The conservation, in seeds plants, of components downstream of this module with MAP kinase target domains, suggests that these proteins are also regulated by these cascades, which, in turn, may be regulated by brassinosteroids and/or ABA.

Contents

1. Introduction

All land plants regulate gas exchange through the stomata, which consist of two kidney-shaped guard cells surrounding a pore. The degree of pore opening is determined by the turgor pressure of the guard cells, which depends on the movement of water and large quantities of ions and sugars between the guard cells and their neighbouring epidermal cells [1]. High turgor pressure induces pore opening, whereas low turgor triggers its closure [1]. The exchange of gases between the plant and the atmosphere depends on the degree of openness of stomatal pore, but also of the number of stomata on the plant surface and of their spatial distribution [2,3].

Stomatal formation in Arabidopsis and other species is preceded by a series of asymmetric cell divisions and a final symmetrical division [4] (Fig. 1). The meristemoid mother cell initiates this process, generating the daughters, meristemoid and stomatal lineage ground cell. The meristemoids can divide asymmetrically (amplifying divisions), and in a spiral pattern, before producing the paired guard cells. Some stomatal lineage ground cells differentiate into pavement cells, whereas others can also divide asymmetrically (spacing divisions) producing new meristemoids (satellite meristemoid) placed away from the pre-existing stoma. This process, which depends on the orientation of the planes of cell division, ensures that the stomata are not in direct contact with their stomata neighbours [4,5], guaranteeing ion flux between guard cells and neighbouring non-stomatal cells, allowing so stomatal movements.

Extensive work carried out during the last two decades supports the existence of a regulatory pathway that controls stomatal development and patterning (Fig. 1). In this pathway, peptides

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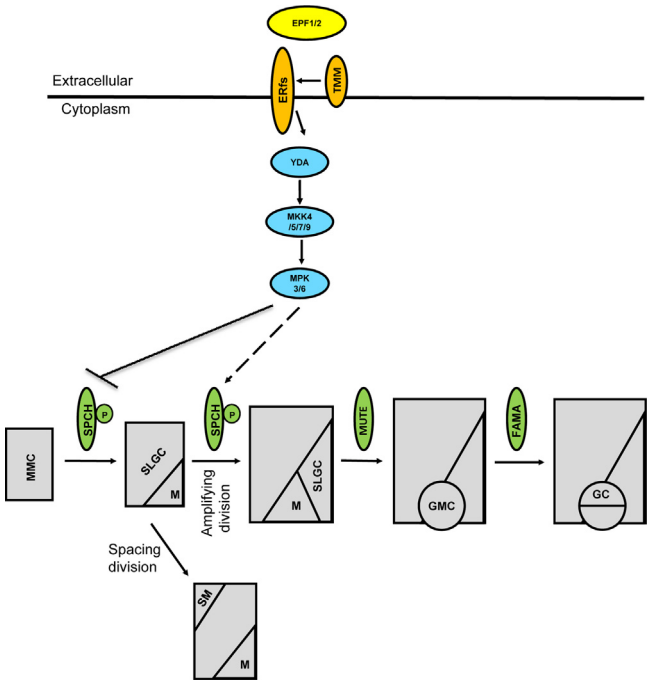

Fig. 1. Schematic diagram of the signalling cascade that controls stomatal development in Arabidopsis. The first step of stomatal development, in which the MMC divides asymmetrically producing the M and the SLGC, is positively regulated by SPCH. SPCH also promotes the asymmetric cell divisions of the Ms (amplifying divisions), which occurs in a spiral pattern. MUTE controls the transition from M to GMC. Finally, FAMA drives the symmetric division of the GMC into two GCs. A MAP kinase signalling cascade, which is activated by TMM and members of the ERfs, which, in turn, are activated by EPF1 and EPF2 ligands, controls SPCH activity. Some SLGCs also divide asymmetrically (spacing divisions) generating SMs, which are placed away from the pre-existing stoma or stomatal precursor.
EPF1, 2, epidermal patterning factor 1 and 2; TMM, too many mouths; ERfs, ERECTA family receptors; YDA, YODA; MKK4, 5, 7, 9, mitogen-activated-protein kinase kinase 4, 5, 7 and 9; MPK3, 6, mitogen-activated-protein kinase 3 and 6; SPCH, SPEECHLESS.
MMC, meristemoid mother cell; M, meristemoid; SLGC, stomatal lineage ground cell; SM, satellite meristemoid; GMC, guard mother cell; GC, guard cell. Dashed arrows indicate hypothetical steps.

ligands of the epidermal patterning factor-like family are recognized by too many mouths (TMM) receptor-like protein, and the receptor-like kinases ERECTA (ER), ERECTA-like1 (ERL1) and ERL2, which allow to the ERECTA-family receptor kinases (ERfs) [4,6,7]. Because TMM associates with ERL1 in vivo [8], the formation of heterodimeric complexes between TMM and ERfs might be required for the initiation of this signalling cascade. In any case, these receptors, activated by peptide ligands, activate a mitogen-activated protein (MAP) kinase module that contains three sequentially activated protein kinases: (1) the MAP kinase kinase kinase YODA; (2) the MAP kinase kinases MKK4, MKK5, MKK7 and MKK9; and (3) the MAP kinases MPK3 and MPK6 [9–11]. MPK3 and MPK6 phosphorylate and destabilize the basic helix-loop–helix protein SPEECHLESS (SPCH), so blocking the entry into the stomatal development pathway [12–14]. SPCH activation drives the first division of stomatal pathway [12]. SPCH not only triggers the initiation of the development of stomata, but also prolongs the cell divisions of the meristemoids [15]. Then, the basic helix-loop-helix MUTE, acting most probably independently of YODA and its downstream kinases [16], blocks the asymmetric cell divisions of the meristemoids, triggering the formation of the guard mother cell [12,13]. Finally, FAMA, which also encodes a basic helix-loop-helix protein [17], causes paired guard cells formation by inducing that guard mother cells divide symmetrically [17].

Stomatal development is regulated by environmental and endogenous factors. Three phytohormones, brassinosteroids, abscisic acid (ABA) and auxins have been implicated in the control of stomatal development [18–21]. This article delves into the role of brassinosteroids and ABA during stomatal development in Arabidopsis and other species. Interestingly, these plant regulators repress stomatal development in Arabidopsis leaves [18–20], affecting the same stages of this process of development, most probably through the regulation of the YODA-MKK4/5/7/9-MPK3/6 module. Indirect evidences also suggest that the regulation of stomatal development through these phytohormones may have arisen after the divergence of lycophytes and seed plants and before that of angiosperms and gymnosperms.

2. Brassinosteroids repress stomatal development in cotyledons and leaves

One of the most well-characterized plant pathways is regulated by brassinosteroids [22,23] (Fig. 2). These phytohormones are perceived by a plasma membrane-localized and leucine-rich-repeat receptor-like kinase named brassinosteroid insensitive 1 (BRI1) [24]. Brassinosteroid recognition by BRI1 results in the activation of a family of kinases named brassinosteroid signalling kinases (BSKs) [25]. Activation of BSKs is followed by the phosphorylation and activation of the Kelch-repeat domain-containing protein phosphatase BRI1 suppressor 1 (BSU1), leading to the dephosphorylation and inactivation of the glycogen synthase kinase3-like kinase brassinosteroid insensitive 2 (BIN2) [25–27]. The repression of BIN2 activity and action of protein phosphatase 2A (PP2A) promote the increase in the nucleus of non-phosphorylated BRI1 EMS suppressor 1 (BES1)/brassinazole resistant 1 (BZR1), regulating many processes such as cell expansion, reproductive development, etiolation, vascular differentiation, cell division and stress responses [28–32]. Without brassinosteroids, BIN2 inhibits BES1/BZR1 function through phosphorylation.

Genetic and pharmacological studies have implicated these plant regulators in stomatal development and patterning. The surface of cotyledons of the quadruple loss-of-function mutant of *BSU1*-related phosphatases (*bsu-q*) was full of paired guard cells, lacking other epidermal cells [18] (Fig. 3B). In spite of this drastic phenotype, *bsu-q* mutant is not lethal. Brassinosteroid biosynthetic (*deetiolated2-1*) or insensitive (*bri1-116*, dominant *bin2-1*, and plants overexpressing *BIN2*) mutant/transgenic plants had cotyledons bearing more stomata than their corresponding wild type plants [18] (Fig. 3C). Some of these stomata were in direct contact with their stomata neighbours [18]. In contrast, the number of stomata was reduced in *deetiolated2-1* plants overexpressing *BSU1- like2* and in plants without brassinosteroid-signalling glycogen synthase kinase3-like kinases (*bin2-3 bil1 bil2* null mutant) [18] (Fig. 3D). These findings support a hypothesis that brassinosteroids negatively regulate stomatal development in cotyledons [18]. As might be expected, plants grown on medium supplemented with either brassinolide (a type of brassinosteroid) or bikinin (a repressor of BIN2 activity) developed less stomata than their controls [18]. Interestingly, brassinosteroids seem to negatively regulate stomatal development in cotyledons independent of the BIN2 substrate BZR1, as gain-of function mutant of *BZR1* had no defect, and this mutant had no effect on stomata formation in *bri1-116*, *bsu-q* and *bin2-1* [18].

The effect of these phytohormones is not limited to cotyledons. Brassinosteroids also repress development of stomatal clusters in leaves. Plants that either lacked brassinosteroids (*constitutive photomorphogenesis and dwarfism*) or had reduced sensitivity to these regulators (*bri1-1*, dominant *bin2-1* and wild type plants overexpressing a glycogen synthase kinase3/shaggy-like kinase

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