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Establishing asymmetry: stomatal division and differentiation in plants

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Summary

In the leaf epidermis, stomatal pores allow gas exchange between plants and the environment. The production of stomatal guard cells requires the lineage cells to divide asymmetrically. In this Insight review, we describe an emerging picture of how intrinsic molecules drive stomatal asymmetric cell division in multidimensions, from transcriptional activities in the nucleus to the dynamic assembly of the polarity complex at the cell cortex. Given the significant roles of stomatal activity in plant responses to environmental changes, we incorporate recent advances in external cues feeding into the regulation of core molecular machinery required for stomatal development. The work we discuss here is mainly based on the dicot plant *Arabidopsis thaliana* with summaries of recent progress in the monocots.

Keywords

Asymmetric cell division; Stomatal development; Cell-division potential; Cell-fate decision; Transcription factors; Cell polarity; Polarity scaffold proteins

I. Introduction

Stomata are turgor-driven microscopic pores in higher plants. The controlled stomatal movement allows efficient gas exchange, restricts excessive water evaporation, and defends pathogen egress. The formation of stomatal complexes in both dicot and monocot plants requires tightly regulated lineage specification, asymmetric cell division, cell-fate transition, and cell-fate differentiation, all of which are programmed by intrinsic developmental pathways and influenced by extrinsic environmental changes.

In dicot plant *Arabidopsis*, the specification of the stomatal lineage cells in young seedlings occurs largely randomly, involving the conversion of a subset of protodermal cells into meristemoid mother cells (MMCs). One MMC divides asymmetrically to produce two

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daughter cells. The small daughter cell becomes a meristemoid and the large one becomes a stomatal lineage ground cell (SLGC), each of which might undergo additional asymmetric cell divisions (ACDs) before terminally differentiating into stomatal guard cells and pavement cells, respectively. The meristemoids often undergo successive self-renewing ACDs in an inward spiral manner, whilst the SLGCs divide asymmetrically to place the newly generated guard cells away from the existing ones (Fig. 1a).

In many monocot plants, stomatal guard cell complexes (two guard cells companioned by two subsidiary cells) are linearly aligned along the longitudinal axis. At the leaf base, stomatal precursor cells divide asymmetrically to produce a small guard mother cell (GMC) that differentiates into guard cells and a large daughter cell that differentiates into a pavement cell. The two neighboring epidermal cells lateral to GMC are recruited to become subsidiary mother cells (SMCs), each of which divides asymmetrically to produce a subsidiary cell adjacent to GMC. The subsidiary cells intimately connected to the GCs enable the four-celled stomatal complexes to move more efficiently for gas exchange (Hepworth *et al.*, 2018; Nunes *et al.*, 2020) (Fig. 1b).

II. Significance of ACD in stomatal development and patterning

In multicellular organisms, stem cells undergo asymmetric cell division (ACD) for self-renewal and generation of daughter cells with specialized identity and function. Stem cell ACD is fundamentally important for plant development, given that organ morphogenesis and patterning mainly occur post-embryonically in plants and require continuous generation of new cell types throughout the whole lifespan. Stomatal ACD is controlled by both cell-autonomous and non-cell-autonomous mechanisms to ensure controlled cell-fate specification, proper distribution, and patterning. The initiation of stomatal lineage cells involves a positive feedback loop of the basic helix-loop-helix (bHLH) transcription factor SPEECHLESS (SPCH) with its partners, the bHLH SCREAMs (SCRM/ICE1 and SCRM2) (Horst *et al.*, 2015). The cell-autonomous function of SPCH as a transcription factor is manifested by its direct binding to the promoters of key genes required for cell-fate transition and differentiation in stomatal development (Fig. 2a and 2b) (Lau *et al.*, 2014). SPCH also drives the expression of the intrinsic polarity factors, BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) and POLAR, which regulate division orientation and cell-fate specification in stomatal ACD (Dong *et al.*, 2009; Pillitteri *et al.*, 2011; Lau *et al.*, 2014). Targets of SPCH and SPCH itself are regulated by peptide- and phytohormone-mediated non-cell-autonomous pathways that contribute to the modulation of stomatal production in response to changing environments (Lau *et al.*, 2014).

III. The SPEECHLESS transcription factor initiates stomatal ACD

The absence of *SPCH* leads to an epidermis devoid of stomata, while elevated protein levels of SPCH result in the over proliferation of stomatal lineage cells in *Arabidopsis* (MacAlister *et al.*, 2007; Lampard *et al.*, 2008). Given the pivotal roles of SPCH in stomatal production, the expression and activity levels of SPCH are tightly modulated at the transcriptional and post-transcriptional levels (Fig. 2b). Red light induces the expression of the LLM-domain B-class *GATA* genes that act upstream of *SPCH* to promote stomatal

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production (Klermund *et al.*, 2016). Also, high-temperature signaling induces the expression of the bHLH transcription factor *PHYTOCHROMEINTERACTING FACTOR 4* (*PIF4*) that binds to the E-boxes within the *SPCH* promoter to suppress *SPCH* expression. In turn, SPCH directly binds to the *PIF4* promoter to suppress *PIF4* expression (Lau *et al.*, 2018). The negative feedback loop of PIF4-SPCH can quickly lower SPCH expression to reduce stomatal production, thereby restricting water loss from plants under warmer conditions. Furthermore, the expression of *SPCH* was found directly inhibited by a C2H2 zinc finger transcription factor INDETERMINATE DOMAIN (IDD) 16 that functions in plant organ morphogenesis (Qi *et al.*, 2019).

At the post-transcriptional level, the stability and function of SPCH proteins are heavily influenced by phosphorylation and de-phosphorylation (Fig. 2b). The Mitogen-Activated Protein Kinase (MAPK) cascade, composed of the MAPKK Kinase YODA (YDA), MAPK Kinase 4 and 5 (MKK4/5), and MAPK 3 and 6 (MPK3/6) in *Arabidopsis*, transduces signals from the cell-surface receptors, including TOO MANY MOUTHS (TMM) and the ERECTA family (Nadeau & Sack, 2002; Shpak *et al.*, 2005). Activated MAPKs trigger an increased level of SPCH phosphorylation for degradation, thus conferring a strong inhibition on stomatal production (Bergmann *et al.*, 2004; Wang *et al.*, 2007; Lampard *et al.*, 2008). Recently, the bHLH partners, ICE1/SCRM and SCRM2, were found to scaffold MPK3/6 into proximity to SPCH (Putarjunan *et al.*, 2019). In addition, the GSK3-like BRASSINOSTEROID INSENSITIVE 2 (BIN2) kinase, a key regulator in Brassinosteroid signaling, phosphorylates SPCH for degradation (Gudesblat *et al.*, 2012). On the other hand, the stabilization of SPCH involves Sucrose non-fermenting-1 (SNF1)-related kinase 1 (SnRK1)- and CYCLIN-DEPENDENT KINASEs A(CDKA;1)-mediated protein phosphorylation (Yang *et al.*, 2015; Han *et al.*, 2020) and Protein Phosphatase 2A (PP2A)-mediated dephosphorylation (Bian *et al.*, 2020). Interestingly, the activity of SPCH in promoting stomatal development requires phosphorylation of the Serine 186 residue that can be modified by BIN2, MPK3/6, or CDKA;1 (Yang *et al.*, 2015). Thus, there is an elegant balance of phosphorylation and dephosphorylation underlying SPCH protein stability and functionality for plants to optimize stomatal production in changing developmental and growth conditions.

The orthologs of *SPCH* have been identified and characterized in monocots. There are two *SPCH* genes in rice and *Brachypodium* and mutant analyses suggested that *SPCH1* and *SPCH2* function largely redundantly for the initiation of the stomatal lineage in both plant species (Raissig *et al.*, 2016; Wu *et al.*, 2019). As in *Arabidopsis*, these SPCHs may also heterodimerize with the partner ICE1/SCRM in monocots (Raissig *et al.*, 2016; Wu *et al.*, 2019). Interestingly, monocot SPCHs together with ICE1/SCRM were recently suggested to partner with the SHORT ROOT(SHR) /SCARECROW(SCR) module to regulate stomatal development (Wu *et al.*, 2019), hinting that mobile SHR may commonly serve as a positional cue derived from the vasculature to assist asymmetric division of outer cell layers in plants.

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IV. The fate of small daughter cell – the meristemoid

After a stomatal ACD in *Arabidopsis*, the small daughter cell meristemoid is projected to differentiate into stomatal guard cells (Fig. 2a). Prior to terminal differentiation, the meristemoid undergoes a few cycles of self-renewing ACD, a process fine-tuned by an antagonistic regulation of ethylene and glucose signaling (Gong *et al*., 2021). The expression of MUTE in late meristemoids switches on the one-time symmetric cell division of GMC by promoting *CYCD5* and possibly *CYCD7*, too (Pillitteri *et al*., 2007; Han *et al*., 2018; Weimer *et al*., 2018). Towards stomatal differentiation, MUTE directly binds to the promoters and activates the expression of *FAMA* and *FOUR LIPS* (*FLP*) (Han et al., 2018), the key transcription factors for terminal differentiation of stomatal guard cells (Lai *et al*., 2005; Ohashi-Ito & Bergmann, 2006) (Fig. 2c). It remains unknown how *MUTE*, potentially a direct target of SPCH, is only expressed in late meristemoids but not as broadly as *SPCH* in early meristemoids and SLGCs. However, how *MUTE* is restricted from excessive expression in GMCs can be explained by the proposed autocrine regulation between MUTE and the peptide-ligand signaling (Qi *et al*., 2017) (Fig. 2c). In this model, MUTE directly induces the expression of the *ERECTA-like 1* (*ERL1*) receptor-like kinase that perceives signaling of the secreted peptide EPIDERMAL PATTERNING FACTOR 1 (EPF1). This peptide-ligand signaling, in turn, triggers the canonical YDA MAPK cascade to suppress nuclear factors, likely including MUTE, in stomatal differentiation (Qi *et al*., 2017) (Fig. 2c). The divisional behavior of the meristemoid is also regulated by auxin signaling because mutations in the auxin efflux transporters resulted in abnormal stomatal differentiation (Le *et al*., 2014). Also, the acquisition of GMC fate is presaged by the PIN3-mediated depletion of auxin levels from the meristemoid but the underlying mechanism and possible connection with MUTE remain obscure (Le *et al*., 2014).

In monocots, ZmMUTE (maize) and OsMUTE (rice) showed conserved regulation of GMC identity, like AtMUTE in *Arabidopsis*. These *mute* mutants fail to produce GMC symmetric division and normal stomatal complexes (Wang *et al*., 2019; Wu *et al*., 2019). Interestingly, the grass MUTE proteins may not be restricted to the GMCs, as the YFP-tagged BdMUTE was found to diffuse into the neighboring subsidiary mother cells (SMCs) (Raissig *et al*., 2017; Wang *et al*., 2019) (Fig. 1b). Accordingly, in *Brachypodium*, the absence of *MUTE* results in the loss of SMC identity, the integrity of the four-celled stomatal complex, and regular stomatal movement (Raissig *et al*., 2017; Wang *et al*., 2019). Thus, MUTE in grasses appears to function autonomously to specify GMC fate and non-autonomously to specify SMC fate.

V. Differentiation of large daughter cell – the SLGC

After a stomatal ACD in *Arabidopsis*, the large daughter cell SLGC is distinguishingly marked by the presence of a polarity module comprised of a few scaffold proteins, including BASL, POLAR, and the BREVIS RADIX (BRX) family (Dong *et al*., 2009; Pillitteri *et al*., 2011; Rowe *et al*., 2019). The SLGCs, relative to the meristemoids, have more restricted cell-division potential and are projected to exit from stomatal fate to ultimately become pavement cells. Recently, Ho et al. employed transcriptional profiling of the polarized BRXL2-enriched SLGCs and identified the chromatin architectural protein DEK and the

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transcription factor MYB16, both of which contribute to the latent status of SLGC between cell division and endoreduplication (Ho *et al.*, 2021). In addition, asymmetric divisions of SLGCs are fine-tuned by feedback crosstalk between SPCH and the cytokinin (CK) signaling components (Vatén *et al.*, 2018).

The restricted division potential of SLGCs is also enforced by polarized BASL that by directly interacting with YDA enriches a "SPCH-targeting MAPK signaling module" (Zhang *et al.*, 2015; Zhang *et al.*, 2016). Thus, the SLGCs differ from the meristemoids by increased levels of MAPK signaling, resulting in elevated SPCH phosphorylation thus lower SPCH protein abundance in the SLGCs. The BRX proteins become polarized in a BASL-interdependent manner and the palmitoylation of BRX helps to tether the BRX-BASL complex to the plasma membrane (Rowe *et al.*, 2019) (Preprint). In the root, BRX interacts with PROTEIN KINASE ASSOCIATED WITH BRX (PAX) to polarly accumulate to the rootward end of developing protophloem sieve elements, where BRX and PAX function as a molecular rheostat to modulate auxin flux and the timing of protophloem differentiation (Marhava *et al.*, 2018). Whether the levels of auxin signaling in SLGCs are similarly modulated by BRX in stomatal development remains unknown.

In *Brachypodium*, despite two copies of *YDA* were identified, mutating *BdYDA1* only was sufficient to give rise to severe stomatal clusters and abnormal recruitment of subsidiary cells (Abrash *et al.*, 2018). Interestingly, BdYDA1-YFP showed polar accumulation in some of the stomatal lineage cells, most frequently at the interface between GMC and the prospective SMC (Abrash *et al.*, 2018). Given the fact that *Brachypodium* does not have *BASL,* whether the four BdBRX proteins contribute to the polarization of YDA should be investigated in the future. Previously in maize, the asymmetric division of SMC was found to be driven by the polarized receptor-like protein PANGLOSS 1 (PAN1) that acts with the Rho of Plants (ROP) small GTPases to form an actin patch in SMC adjacent to GMC (Cartwright *et al.*, 2009; Humphries *et al.*, 2011; Facette *et al.*, 2015) (Fig. 1b). It is worth noting that the polarity site in both systems (maize and *Arabidopsis*) plays roles in directional nuclear migration and division orientation in developing stomata. However, not much has been known, particularly in *Arabidopsis*, about how polarity proteins impinge on the spatial organization of the cytoskeleton elements and/or drive directional sometimes opposite nuclear migration before and after an ACD (Muroyama *et al.*, 2020).

VI. Timed assembly of polarity components – before, during, and after stomatal ACD

The polarity platform represented by BASL was found to maintain before, during, and after a stomatal ACD (Dong *et al.*, 2009). However, the ACD mother cell (MMC) fundamentally differs from the daughter cell (SLGC) with regards to cell-division potential (high in MMC and low in SLGC, Fig. 3). Although both contain the seemingly "same" polarity complex, the MMC expresses high levels of SPCH and undergoes more active stem-cell-like division, whereas the SLGC contains low levels of SPCH and is restricted from active cell division (Fig. 3). The distinct activities of MMC and SLGC might result from the distinct signaling

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components and functions that are dynamically organized by the polarity platform before, during, and after an ACD, respectively.

More specifically, the YDA MAPK signaling cassette associates with BASL polarity in both MMC and SLGC. The positive feedback between BASL and the YDA MAPK signaling confers a strong inhibition on the division-promoting activity of SPCH in SLGC (Zhang *et al.*, 2015) (Fig. 3c). However, the suppression on SPCH must be alleviated to allow high division potential in MMC. It was recently reported that the GSK3-like BIN2 kinases are recruited to the polarity site through direct interaction with POLAR (Houbaert *et al.*, 2018). Therefore, in MMC, the cortically enriched BIN2 can inhibit YDA kinase activity, thereby releasing the YDA MAPK-mediated inhibition to allow SPCH to accumulate in MMC (Kim *et al.*, 2012; Houbaert *et al.*, 2018) (Fig. 3a). Thus, although the YDA MAPK cascade is polarized in both MMC and SLGC, the participation of BIN2 in the polarity site suppresses YDA to maintain the high division potential of MMC(Guo & Dong, 2019).

Then, the next question was how the inhibition of BIN2 on YDA can be alleviated after division in SLGC. Recently, members of the BRI1 SUPPRESSOR (BSU1) family were identified to interact with BASL and the founding member BSL1 joins the polarity site and functions as a molecular switch to enable the transition from MMC to SLGC (Guo *et al.*, 2021). Time-lapse examination of fluorescent protein-tagged BSL1 showed that BSL1 becomes polarized slightly later than BASL in MMC and coincides with the formation of the preprophase band (an indication of the entry to mitosis) (Fig. 3b). The association of BSL1 with the polarity complex on one hand leads to the translocation of BIN2 from the plasma membrane to the nucleus, likely due to its dephosphorylation activity. Consequently, the BIN2-mediated inhibition of YDA is released, whilst nuclear BIN2 may directly bind and phosphorylate SPCH for degradation (Gudesblat *et al.*, 2012). On the other hand, polarized BSL1 directly binds to YDA for dephosphorylation and activation, leading to elevated MAPK signaling and further suppressed SPCH (Guo *et al.*, 2021). Therefore, through the joint regulation of BIN2 (translocation) and YDA (activation), the assembly of BSL1 in the polarity complex, upon the MMC entry to mitosis, can quickly lower the protein level of SPCH and tune down the division potential of MMC, thereby enabling the progression towards SLGC in stomatal ACD (Guo *et al.*, 2021).

VII. Outstanding questions

Stomatal development has proven to provide an excellent platform for dissecting molecular mechanisms underlying coordinated activities of cell polarity, self-renewing division, and cell-fate differentiation in plant development. A few outstanding questions remain in the field. First, surrounding the master regulator SPCH, is there a phosphocode-dependent regulatory dichotomy of the SPCH protein in degradation and activity? How is MUTE, the terminator of stomatal ACD, tightly restricted to express in a specific population of meristemoids? Does this process involve chromatin remodeling as suggested by (Lee *et al.*, 2019)? How grass MUTE proteins can travel and induce the formation of the specialized subsidiary cell is also a big open area. The fast-expanding list of polarized proteins participating in the regulation of *Arabidopsis* stomatal ACD raised a long-standing question - whether a complementary polarity domain exists and contributes to the maintenance of

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cell polarity. It was also clear that, although the BASL polarity site is largely defined by intrinsic mechanisms (Chan *et al.*, 2020), the mechanism for polarity switch during SLGC spacing division, presumably driven by signals released from the neighboring GMC, remains unknown. Also, YDA was found to commonly polarize in both monocot and dicot stomatal ACD cells. How YDA becomes polarized in the grasses and whether YDA polarization overlaps with the PAN signaling module are all uncharacterized.

The application of newer technologies, such as CRISPR-Cas-aided reverse genetics, cell-type-specific knocking out essential genes, and TurboID-based proximity labeling combined with quantitative proteomics, *etc.*, will be greatly helpful for identifying new regulators and better characterizing known regulators *in vivo*. Successful basic research creates new directions and will ultimately lead to the pathways to produce plants with improved traits for agricultural applications.

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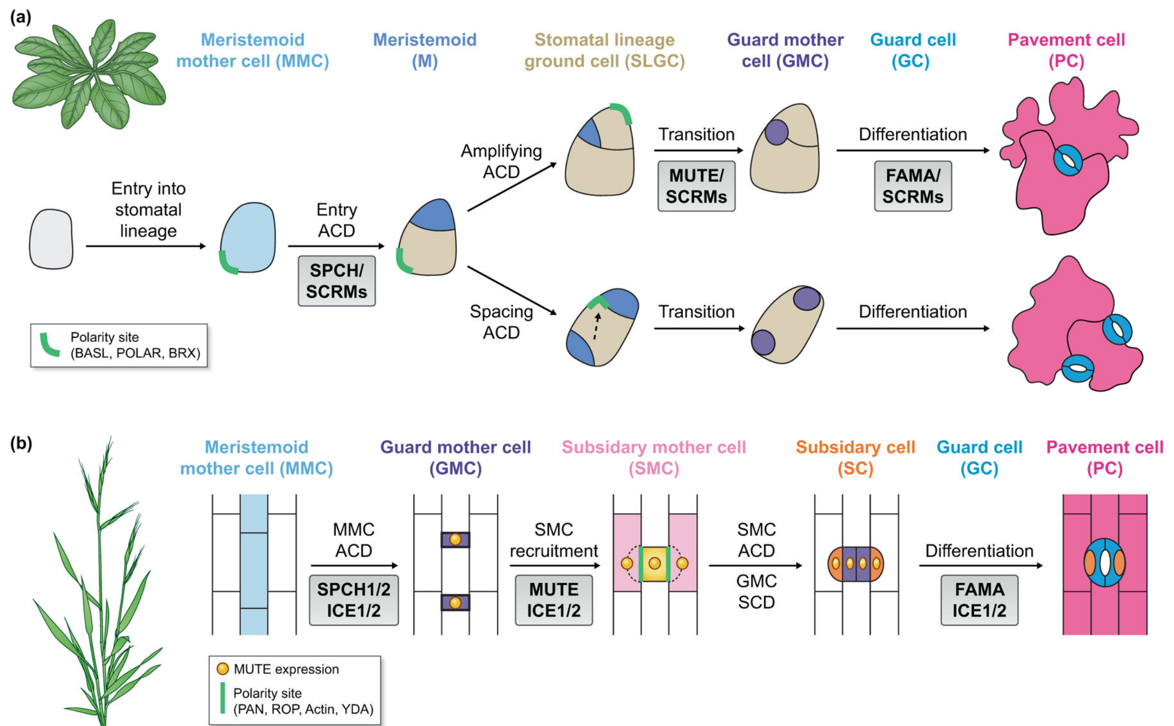

Fig. 1. Stomatal division and differentiation in dicots and monocots.

Diagrams show cell types, asymmetric cell division (ACD) and stomatal differentiation progresses in *Arabidopsis* (a, example for dicots) and *Brachypoium* (b, example for monocots). Key regulators for successive cell-fate transitions are specified in grey boxes. Particularly interesting cellular events (polarity site and mobile transcription factor MUTE) are highlighted accordingly.

(a) In *Arabidopsis*, SPCH and SCRMs initiate stomatal asymmetric division of the meristemoid mother cell (MMC) (light blue) to produce a small daughter cell meristemoid (M) (blue) and a large daughter cell, stomatal lineage ground cell (SLGC) (pink). The polarity complex (green) (assembled by scaffold proteins BASL, POLAR, and BRX) is expressed in MMC and SLGC. After a few rounds of meristemoid amplifying ACD, MUTE enables cell-fate transition to guard mother cell (GMC) (purple). FAMA then promotes cell-fate differentiation to guard cell (GC) (dark blue). The SLGC spacing division involves directional switch of the polarity site (dashed arrow) and generates a satellite meristemoid distal to the polarity site.

(b) In *Brachypodium*, the formation of both guard cells (dark blue) and subsidiary cells (SC) (red) require ACD (MMC ACD and SMC ACD, respectively). Key regulators and possible partners of cell-fate determination (grey boxes) are summarized from research in *Brachypodium*, rice and maize. In grasses, the expression of SPCHs in MMC (light blue) may partner with ICE1 and ICE2 to initiate entry ACD that produces a small daughter cell (GMC, purple) and a large daughter pavement cell (PC) (light pink). The formation of GMC induces the recruitment of adjacent subsidiary mother cells (SMCs) (pink) and direct the orientation of SMC ACD. The expression of MUTE (yellow) in GMC specifies the GC fate (dark blue). MUTE also moves out of GMC to recruit SMC and specify the SC fate. The polarity proteins (green) include the PAN receptor-like proteins that recruit Type I ROP

GTPases to the SMC surface contacting nascent GMCs. ROPs induce the polarization of F-actin regulators, which promote both the polarization of PAN polarization and directional nuclear migration in SMC. YDA has a predominant role in the suppression of stomatal formation and shows polar accumulation at the interface between GMC and SMC.

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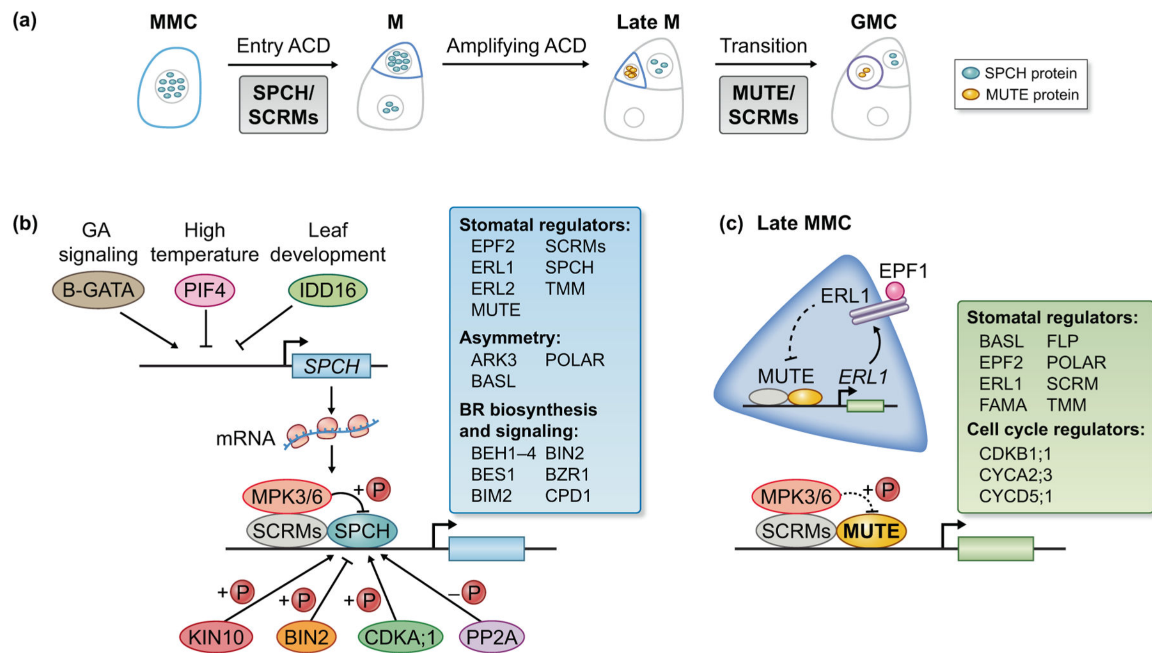

Fig. 2. Regulatory networks of SPCH and MUTE in *Arabidopsis* stomatal development.

(a) Diagrams depict the protein expression levels of SPCH (blue ovals) and MUTE (yellow ovals) in stem-cell-like stomatal ACD. The density of ovals represents protein amount.

(b) Regulatory networks around SPCH. Upstream transcription factors responding to developmental or environmental cues can regulate *SPCH* transcription. PIF4 in the high temperature signaling pathway and IDD16 in the organ morphogenesis pathway may directly bind to the *SPCH* promoter and suppress *SPCH* expression, whilst B-GATA in GA signaling promotes *SPCH* expression. At the protein level, SPCH is tightly regulated by phosphorylation and dephosphorylation. The bHLH transcription factors, ICE1 and SCRM2, bring MPK3/6 close to SPCH for phosphorylation. MPK3/6- and BIN2-mediated phosphorylation promotes SPCH degradation, whereas the CDKA;1 and KIN10 kinase-mediated phosphorylation and the phosphatase PP2A-mediated dephosphorylation promote SPCH stabilization. CDKA;1 also phosphorylates SPCH for activation. Downstream of SPCH, based on the work from (Lau *et al*., 2014), SPCH directly regulates the expression of key genes in stomatal development, divisional asymmetry, and hormonal signaling, particularly BR biosynthesis and signaling (blue box).

(c) Regulatory networks around MUTE. MUTE (yellow) is expressed in late meristemoids and promotes the cell-fate transition to GMC. Based on the work from (Han *et al*., 2018), MUTE directly controls the key genes functioning in stomatal development and cell-cycle control (green box). An autocrine signaling regulation (diagram on the left) was proposed to contribute to the restricted expression of MUTE in late meristemoids. MUTE directly induces the expression of ERL1 that perceives signaling of the secreted peptide EPF1. Downstream of ERL1, the YDA MAPK cascade was suspected to inhibit MUTE function (dashed arrow).

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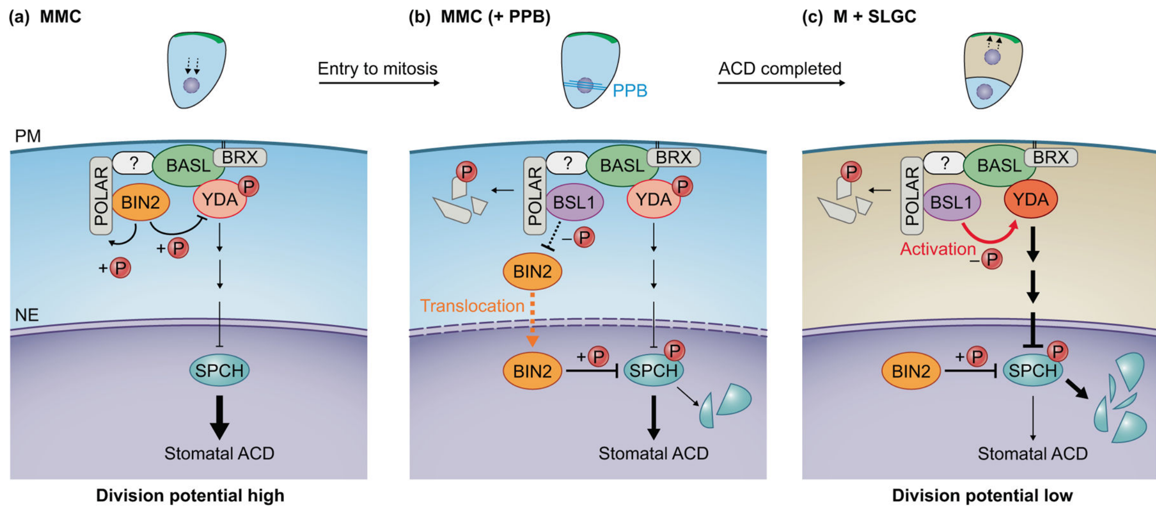

Fig. 3. Timed assembly of polarity components during stomatal ACD

Diagrams on top show subsequent events during a stomatal asymmetric cell division (ACD) (a-c). The formation of the preprophase band (PPB, blue lines) indicates the cell entering mitosis. The direction of nuclear migration (dashed arrows) was found against and towards the polarity site, before and after the division, respective. Diagrams below detail the specific changes in the components of the polarity complex leading to differential division potential of the cell at different stages.

(a) Before an ACD (in MMC, meristemoid mother cell), BASL (green) is polarized at the cell cortex through the interaction with BRX that is palmitoylated to attach to the plasma membrane (grey). Polarization of POLAR (grey) is largely dependent on BASL, but its association with the polarity complex may need an unidentified component (box with a question mark). Polarized POLAR recruits the BIN2 GSK3-like kinases (orange) to inhibit the kinase activity of YDA (pink), leading to alleviated MAPK-mediated suppression on SPCH (blue). A high level of SPCH sustains a high level of cell-division potential in the MMCs. PM, plasma membrane. NE, nuclear envelope.

(b) During an ACD, BSL1 (purple) associates with the BASL polarity complex. The polarization of BSL1 coincides with the formation of the PPB upon MMC entering mitosis. Polarized BSL1 inhibits BIN2's function at the PM involving the BSL1 phosphatase activity and mainly through dissociating BIN2 from the PM. Consequently, the BIN2 inhibition on YDA is released, leading to elevated SPCH degradation (broken blue oval). At the PM, BIN2 may trigger the turnover of POLAR through phosphorylation (broken grey box) that lowers the PM-association of BIN2. When BIN2 is enriched in the nucleus, it promotes SPCH degradation *via* phosphorylation.

(c) After an ACD, the BSL1-BASL-YDA polarity complex is inherited by SLGC (stomatal lineage ground cell). BSL1 directly activates YDA through dephosphorylation, so that elevated MAPK signaling confers strong suppression on SPCH and lowered cell-division potential. Therefore, the participation of BSL1 in the polarity complex during the transition from MMC to SLGC jointly regulates BIN2 localization and YDA activity, enabling the transition from high division potential to low division potential in MMC and SLGC,

respectively. This process is essential for the progression of stomatal ACD and the specification of the two daughter cells with distinct developmental trajectories.

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