Published in final edited form as:

Trends Plant Sci. 2019 June ; 24(6): 481–484. doi:10.1016/j.tplants.2019.03.007.

To Divide or Differentiate: It Is about Scaffolding

Xiaoyu Guo¹, Juan Dong^{1,2,*}

¹Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA

²Department of Plant Biology, Rutgers, The State University of New Jersey, Piscataway, NJ 08901, USA

Abstract

Arabidopsis plants have specific polarized proteins, BASL and POLAR, that regulate stomatal asymmetric cell division (ACD). Earlier work established that BASL recruits YODA kinase (MAPKKK4) to suppress stomatal ACD in postmitotic cells. Houbaert *et al.* (*Nature* 2018;563:574–578) recently showed that POLAR scaffolds BIN2/GSK3s to promote stomatal ACD in premitotic cells.

Stomata are microscopic pores in the epidermis of aerial organs that regulate gas exchange between plants and the atmosphere. The patterning and production of stomata in the model plant arabidopsis (*Arabidopsis thaliana*) require sequential rounds of stem cell-like asymmetric cell division (ACD) and differential cell fate specification. These processes are initiated by the key bHLH transcription factor SPEECHLESS (SPCH), and extra levels of SPCH induce stomata overproduction [1,2]. The abundance of SPCH is negatively regulated by protein phosphorylation mediated by enzymes including mitogen-activated protein kinases (MAPKs) and BIN2, a glycogen synthase kinase 3 (GSK3)-like kinase [2,3].

A stomatal precursor cell, the meristemoid mother cell (MMC), divides asymmetrically to produce a small meristemoid (M) cell and a large stomatal lineage ground cell (SLGC), and the two daughter cells take on distinct developmental paths (Figure 1A). The M has high ACD potential and, after a few rounds of amplifying divisions, becomes a guard mother cell (GMC) that divides symmetrically and terminates in highly differentiated guard cells (GCs). The SLGC either expands to become a pavement cell (PC) or, with limited division potential, undergoes an ACD to space out the newly formed M. SPCH levels were found to be high in ACD-active cells, for example MMCs and Ms, but were low in SLGCs [4].

Similar to the animal ACD systems, for example neuroblasts in *Drosophila melanogaster* and embryos in *Caenorhabditis elegans*, the stomatal lineage cells in arabidopsis exploit cortically polarized proteins, including BREAKAGE OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) [5] and POLAR [6]. Absence of *BASL* leads to stomatal divisions that are more symmetric [5]. BASL polarization requires MAPK-mediated phosphorylation, and polarized BASL, in turn, recruits the MAPKK kinase YODA to the

*Correspondence: dong@waksman.rutgers.edu (J. Dong).

polarity site, leading to elevated MAPK signaling that promotes more BASL polarization. This positive feedback loop amplifies MAPK signaling, giving rise to stronger suppression of SPCH, and thus to reduced SLGC division potential [7]. POLAR was identified by expression profiling of stomatal ACD cells, and its polarization is dependent on BASL, but no direct physical interactions were detected [6]. Establishing how POLAR regulates stomatal ACD has been hampered by the absence of discernable growth and developmental defects in *polar* loss-of-function single mutants [6]. An additional mystery was that BASL/POLAR polarity appears both pre- and postmitotically, suggesting that the same polarity complex has opposing functions in both promoting and restricting stomatal ACD. This major functional discrepancy was recently reconciled by a breakthrough study that established the scaffolding function of POLAR in premitotic stomatal lineage cells, where POLAR recruits BIN2/GSK3s to suppress YODA MAPK signaling activity in the polarity complex [4].

The GSK3-like Kinase BIN2 in Brassinosteroid (BR) Signaling

The GSK3/SHAGGY kinases are a group of highly conserved serine/threonine kinases that are implicated in numerous signaling pathways in eukaryotes. The arabidopsis genome encodes 10 GSK3-like kinases, and the founding member, BIN2, plays a pivotal function in suppressing BR signaling [8]. In the absence of BR, BIN2 constitutively inhibits two redundant transcription factors, BES1 and BZR1, that activate BR responses [8]. In the presence of BR, the receptor-like kinases BRI1/BAK1 are activated, followed by signal transduction that eventually releases suppression by BIN2, and thus triggers BR responses [9].

Positive or Negative? The Puzzling Function of BIN2 in Stomatal Development

BIN2 regulates stomatal development in a more complex manner [3,10]. On the one hand, BIN2 may function as a positive regulator by inhibiting the MAPKKK YODA and MKK4/5, resulting in more accumulation of SPCH, and thus more stomatal production [10]. On the other hand, BIN2 also functions as a negative regulator by directly phosphorylating and destabilizing SPCH [3]. The seemingly contradictory effects of BR signaling and BIN2 in stomatal development were suspected to reflect different experimental conditions and/or organ-specific regulation in plants [11]. However, new evidence suggests that sophisticated functional regulation of BIN2 activity occurs at the subcellular level.

In contrast to its explicit genetic roles in BR signaling, the subcellular localization of BIN2 was somewhat ambiguous in earlier studies. Current understanding is that BIN2 is localized in the cytoplasm and nucleus, and that shuttling between these two subcellular locations might be crucial for its function in BR responses. In addition, the functional complexity of BIN2 kinase is reflected by its wide range of substrates that include several nuclear transcription factors (BZR1/BES1, MYBL2/HAT1, and SPCH, etc.) and cytoplasmic factors, such as the SnRK2 protein kinases and the ABI1/2 protein phosphatases. These studies suggested that the signaling specificity of BIN2 might be largely controlled by its subcellular partnership with another regulatory factor.

The Puzzle Solved: Differential Subcellular Partitioning of BIN2 in Stomatal ACD

In stomatal development, the two apparently contradictory phenomena, the positive and negative roles of the BASL/POLAR polarity in stomatal ACD, as well as the positive and negative roles of BIN2 in stomatal production, can now be explained by the establishment of POLAR as a scaffold protein that spatially modulates the nuclear–cytoplasmic partitioning of BIN2 in stomatal lineage cells [4]. Houbaert *et al.* identified the polarity protein POLAR as a BIN2-associated protein, and showed that POLAR accumulates BIN2 and a few other GSK3-like kinases at the cortical polarity site in the stomatal ACD precursors, MMCs, resulting in more cytoplasmic and less nuclear partitioning of BIN2/GSK3s in these cells (Figure 1B). This leads to the preferential cytoplasmic function of BIN2/GSK3s in suppressing YODA and MKK4/5, but reduced nuclear function in suppressing SPCH. Thus, the collective effects result in high SPCH levels in the MMCs, such that stomatal ACD is promoted.

However, the polarization of BIN2 in the MMCs appears to be transient, and disappears in SLGCs after stomatal ACD, whereas BASL polarity remains strong. How BIN2 dissociates from the polarity complex is largely unknown, but Houbaert *et al.* suggested that this can be achieved by negative feedback regulation from BIN2 to POLAR. *In vitro* kinase assays detected BIN2-mediated phosphorylation of POLAR, and mutagenesis of POLAR suggested that the participation of BIN2 in the polarity complex triggers faster turnover of POLAR, which in turn negatively impacts on BIN2 association with the polarity complex. This has two consequences in SLGCs: (i) BIN2-mediated inhibition of the YODA MAPK module is released such that the positive feedback loop of BASL–YODA–MPK3/6 is enabled and SPCH is suppressed; and (ii) increased enrichment of BIN2 in the nucleus enhances SPCH phosphorylation and degradation. Therefore, the combined effects significantly reduce SPCH protein levels, thus reducing the ACD potential of SLGCs (Figure 1B).

Concluding Remarks and Outstanding Questions

In summary, the plant-specific, intrinsic polarity proteins BASL and POLAR are now established as scaffold proteins, mirroring the scaffolding function of the Par proteins in animal ACD systems. However, the assembly of the polarity complex in plants appears to be more dynamic, and this is well exemplified by the BASL/POLAR module discussed here. Such dynamic reassembly of the polarity module in the stomatal ACD system may provide flexibility to coordinate complex signaling pathways in balancing cell division and differentiation, thereby fine-tuning the stemness of stomatal lineage cells in arabidopsis to meet the challenges of the ever-changing environment.

One lesson we have learned from studying BIN2 is that careful cell-biological analysis is necessary for better characterization of such multifunctional signaling molecules. For example, BIN2 can be a positive or negative regulator in the BR-activated phloem differentiation process, depending on the presence or absence of the peripheral membrane protein OCTOPUS (OPS), respectively [12]. Where and when these signaling molecules function might be crucial in elucidating how they regulate a specific pathway.

Although recent discoveries have improved our understanding of cell polarity in the regulation of stomatal ACD, several outstanding questions remain to be addressed.

- i. What is the molecular mechanism underlying protein polarization in the stomatal ACD system? MAPK- and BIN2-mediated phosphorylation may promote BASL/POLAR polarization [4,7], but the mechanisms by which these peripheral membrane proteins are polarly targeted and maintained at the cortical membrane domain remain a major challenge.
- ii. How is the polarity complex differentially assembled at different stages of stomatal ACD? The mechanism underlying the dynamic assembly of BIN2/GSK3s and YODA/MPK3/6 at the polarity site is unknown, and its elucidation would give unique insights into the mechanisms that balance cell division and fate differentiation in plant stem cells.
- iii. Direct physical relationships among the polarity factors (BASL, POLAR, YODA, and BIN2/GSKs) need to be elucidated to construct the dynamic architecture of the polarity modules at different stages. In particular, evidence of physical linkage between BASL and POLAR is still lacking. Moreover, although BIN2/GSK3s suppress POLAR protein stability, positive regulator(s), so far unidentified, are anticipated to promote polarity formation by BASL/POLAR.

Technical advances will be helpful in addressing these questions. For example, single-molecular imaging is a powerful technique that can reveal dynamic protein interactions in living cells. Light-sheet fluorescence microscopy allows long-term and in-depth imaging at single-cell resolution that is particularly effective for studying plant cell development. In addition, the emergence of the proximity labeling-based spatial proteomics holds promise for resolving protein interaction networks that underlie cell signaling processes.

Acknowledgments

Research by the group of J.D. is supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number R01GM109080.

References

1. MacAlister CA et al. (2007) Transcription factor control of asymmetric cell divisions that establish the stomatal lineage. *Nature*, 445, 537–540 [PubMed: 17183265]
2. Lampard GR et al. (2008) Arabidopsis stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. *Science*, 322, 1113–1116 [PubMed: 19008449]
3. Gudesblat GE et al. (2012) SPEECHLESS integrates brassinosteroid and stomata signalling pathways. *Nat. Cell Biol.* 14, 548–554 [PubMed: 22466366]
4. Houbaert A et al. (2018) POLAR-guided signalling complex assembly and localization drive asymmetric cell division. *Nature*, 563, 574–578 [PubMed: 30429609]
5. Dong J et al. (2009) BASL controls asymmetric cell division in Arabidopsis. *Cell*, 137, 1320–1330 [PubMed: 19523675]
6. Pillitteri LJ et al. (2011) Molecular profiling of stomatal meristemoids reveals new component of asymmetric cell division and commonalities among stem cell populations in Arabidopsis. *Plant Cell*, 23, 3260–3275 [PubMed: 21963668]
7. Zhang Y et al. (2015) The BASL polarity protein controls a MAPK signaling feedback loop in asymmetric cell division. *Dev. Cell*, 33, 136–149 [PubMed: 25843888]

8. He JX et al. (2002) The GSK3-like kinase BIN2 phosphorylates and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in Arabidopsis. *Proc. Natl. Acad. Sci. U. S. A* 99, 10185–10190 [PubMed: 12114546]
9. Kim TW et al. (2009) Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. *Nat. Cell Biol* 11, 1254–1260 [PubMed: 19734888]
10. Kim TW et al. (2012) Brassinosteroid regulates stomatal development by GSK3-mediated inhibition of a MAPK pathway. *Nature*, 482, 419–422 [PubMed: 22307275]
11. Gudesblat GE et al. (2012) Brassinosteroids tailor stomatal production to different environments. *Trends Plant Sci.* 17, 685–687 [PubMed: 23022359]
12. Anne P et al. (2015) OCTOPUS negatively regulates BIN2 to control phloem differentiation in Arabidopsis thaliana. *Curr. Biol* 25, 2584–2590 [PubMed: 26387715]

Figure 1. The Dynamic BASL/POLAR Polarity Module in Stomatal Asymmetric Cell Division (ACD).

(A) The BASL/POLAR polarity module (orange crescent) first appears at the cortex of a stomatal precursor meristemoid mother cell (MMC) cell, and, after an ACD, is inherited by the large daughter cell (stomatal lineage ground cell, SLGC). The SLGC may undergo spacing ACD that involves reoriented BASL/POLAR polarity before pavement cell (PC) differentiation. The small daughter cell, meristemoid (M), undergoes a few rounds of ACD before terminal differentiation into a pair of guard cells (GCs). (B) Schematics of the polarity module in stomatal ACD: premitotic BASL/POLAR/BIN2 and postmitotic BASL/POLAR/YODA. Before ACD, BASL recruits BIN2/GSK3s through the scaffold protein POLAR. The cortical enrichment of BIN2/GSK3s inhibits the YODA MAPK cassette by phosphorylating YODA and MKK4/5, leading to elevated accumulation of SPCH that promotes stomatal ACD. Cortical BIN2 also phosphorylates POLAR, resulting in reduced

POLAR accumulation and dissociation of BIN2 from the polarity site, enabling a positive feedback loop between BASL and the YODA MAPKKK cassette in the SLGCs. The combined effects of the BIN2 nuclear enrichment and elevated MAPK signaling induce strong suppression of SPCH, thus leading to restricted stomatal ACD in the SLGCs.