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Cell shape control: a tug-of-war between ROP activators and inhibitors regulates plant cell lobing

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Rho-related GTPase from Plants (ROPs) coordinate the complex morphogenesis of leaf pavement cells. Four new studies reveal how ROP activity is regulated by both activating and inhibitory proteins to orchestrate cell lobing in response to local extracellular cues.

The epidermis is the interface between the plant and its environment and it represents an essential protection layer. Besides, it plays essential roles in the regulation of tissue and organ growth. The pavement cells with their characteristic jigsaw puzzle shape are the fundamental unit of the leaf epidermis¹. The formation of interdigitating undulations is orchestrated by ROP2 and ROP6 that act antagonistically to control cell lobing (Figure 1)². Furthermore, ROP2 and ROP6 act as nodes that integrate cell-intrinsic and extrinsic pathways to regulate cytoskeleton organization and ultimately pavement cell shape³. How ROP2 and ROP6 control cortical actin and microtubule cytoskeletons has been extensively studied (see Figure 1)^{1,3}. However, little is known on the upstream mechanisms that steer ROP spatiotemporal activity in pavement cells. ROPs can cycle between an activated GTP-bound state and an inactivated GDP-bound state². This cycle is coordinated by Guanosine Nucleotide Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs). GEFs facilitate the exchange of GDP-to-GTP, which activate ROP GTPases. GAPs trigger the intrinsic GTPase function of the G-protein, leading to GTP hydrolysis and ROP inactivation. Four new publications in Current Biology uncovered specific GEF and GAP proteins that modulate ROP6 or ROP2 spatiotemporal activation in response to extracellular cues⁴⁻⁷.

In this issue of Current Biology, Zhang et al., and Lauster et al., uncovered PHGAP1 and PHGAP2 (also called ROP-ENHANCER (REN) 2 and 3) as key regulators of cell lobing^{4,7}. The *phgap1phgap2* double mutant has severe pavement cell shape phenotypes, mimicking overexpression of a constitutive active ROP2 mutant (*ca-rop2*). Bimolecular fluorescence complementation experiments previously suggested unspecific binding between PHGAPs and several ROP proteins⁸. However, using coimmunoprecipitation, Lauster et al. demonstrated that PHGAPs specifically interact with ROP2 and not ROP6 *in planta*⁷. Furthermore, activity assays indicated that the *phgap1phgap2* double mutant has an elevated activation of ROP2 but not ROP6 compared to the wild type, hinting for a role of PHGAP1 and PHGAP2 in specific ROP2 inhibition. In line with this finding, microtubule anisotropy in the *phgap1phgap2* double mutant is similar to those of *ca-rop2*. Both studies reveal the remarkable localization of PHGAP1 and PHGAP2 at the anticlinal face of the cell (Figure 1)^{4,7}. Notably, PHGAPs preferentially accumulate in the anticlinal face on the indentation side, where they form stripes at the plasma membrane⁷. This localization pattern strikingly resembles that of the microtubules that populate the cortical anticlinal face in the indentation and then extend to the outer periclinal face of the cell (i.e., transfacial, Figure 1)⁹. Indeed, PHGAPs largely colocalize with microtubules and are dependent on microtubules for their association with the anticlinal face in the indentation region⁷. Thus, PHGAPs are recruited to transfacial microtubules in

indentation, where they inactivate ROP2 thereby delimiting an optimum membrane domain for ROP6 activity (Figure 1).

Zhang et al. found that PHGAPs are themselves regulated by the plant growth hormone brassinosteroids (BRs), through the action of BR-INSENSITIVE2 (BIN2), a negative regulator of BR signaling^{4,10}. BIN2 was previously shown to associate with the plasma membrane in leaf epidermis¹¹ and to interact and stabilize microtubules¹². Now, Zhang et al. uncovered the role of BIN2, downstream of BR signaling, to stabilize PHGAPs at the anticlinal face of the undulations⁴. Indeed, BIN2 is a SHAGGY/GSK3-like kinase (AtSK) that directly binds to and phosphorylates PHGAPs. Chemical inhibition of BIN2 (using BR or bikinin treatment^{10,13}) or mutation of BIN2 phosphorylation sites destabilized PHGAPs, notably in the lobe region (Figure 1). Similarly, genetic ablation of the AtSK family using sextuple or octuple mutants affects leaf pavement cell shape and PHGAP stability⁴. Thus, Zhang et al. proposed that BRs stimulate ROP2 activity in lobes by inducing the local degradation of PHGAPs⁴.

Transfacial microtubule bundles connect the indentation regions of pavement cells, which correspond to zones of predicted maximal tensile stresses^{9,14}. This co-alignment of microtubules with mechanical stress patterns is dependent on the microtubule severing enzyme Katanin (KTN1)¹⁴, which is itself regulated by ROP6¹⁵. Now, two back-to-back papers from Zhenbiao Yang's group found a direct mechanistic link between the pectin status in the cell wall and ROP6 activation^{5,6}. This pathway involves the direct binding of the receptor kinase FERONIA (FER) with demethylesterified pectins⁶. Upon interaction with pectins, FER recruits RopGEF14 (GEF14), which, in turn, activates ROP6 (Figure 1). The authors propose that the pectin-FER-GEF14-ROP6 complex might transduce mechanical forces to control microtubule orientation⁵. However, this idea is contested in another study which put forward a model in which FER action in response to mechanical stress is independent of microtubule reorganization¹⁶. Thus, the link between pectin perception and mechanical transduction remains to be fully elucidated.

Irrespective of a possible role in mechano-transduction, pectins appear to act as key local determinants in cell lobing. Indeed, methylesterified and demethylesterified pectins have distinct mechanical properties and they preferentially accumulate in lobes and indentations, respectively (Figure 1)^{6,17,18}. Of note, the cell wall pectin composition can regulate BR signaling^{19,20}. In particular, pectin imbalance resulting from the inhibition of pectin demethylesterification, which leads to the enrichment of methylesterified pectins, caused the activation of BR signaling²⁰. A localized inhibition of BIN2 in the lobe region downstream of methylesterified pectins could contribute to the spatial regulation of PHGAP localization (figure 1). However, a direct link between BIN2 activity and the pectin status in the cell wall of pavement cells remains to be fully explored.

Altogether, these stories highlight how the local regulation of either GEF or GAP proteins patterns the activity of different ROPs within distinct membrane domains of pavement cells. A fascinating finding in these papers is the selectivity of PHGAPs and GEF14 for ROP2 and ROP6, respectively. Indeed, both ROPs are almost identical in terms of sequences and both are expressed in pavement cells. The mechanistic bases behind the specific actions of GEFs and GAPs on given ROPs remain enigmatic and is an exciting topic for future studies.

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Figure 1: Position of active ROP6 and ROP2 domains via GEF and GAP regulators.

Schematic representation of two juxtaposed pavement cells (Cell 1 and Cell 2) with the position of the active ROP2 (ROP2^{GTP}, light blue) domains in the lobes and active ROP6 (ROP6^{GTP}, light orange) domains in the indentation. Active ROP2 promotes F-actin polymerization and lobe formation, while active ROP6 organizes cortical microtubules in the indentation. Cortical microtubules are bundled together in the anticlinal face of the indentation and then fan out on the periclinal face to connect indentations. They thus span several faces of the pavement cell (i.e., transfacial). The formation of an active ROP6 domains in the indentation region results from the concomitant activation of ROP6 by GEF14 and inhibition of ROP2 by PHGAPs. Asymmetric deposition of differentially methylesterified pectins in the cell wall adjacent to lobes or indentation might also contribute to the local activation of ROP6 or ROP2, via GEF14/PHGAPs regulation. PECTIN^{DME}, demethylesterified pectins; PECTIN^{ME}, methylesterified pectins; BRI1, BR-INSENSITIVE1 (BR receptor).

