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# Assorted Pastries at the South Pole

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**Plant cells use polarity cues to confine membrane proteins to specific localizations. In this issue of *Developmental Cell*, Marhava et al. (2020) describe a biochemical feedforward mechanism that reinforces polar protein localization and regulates membrane composition and endocytosis.**

Cells in multicellular organisms often have shapes, appendages, or functions that are non-uniform and differ between their faces. Such anisotropy—or polarity—is an important component for organizing multicellular life in 3D and is critical for many cellular functions, such as maintaining epithelial integrity, arranging hairs and bristles, and orienting cell-division planes (St Johnston and Ahringer, 2010). Cell polarization involves the local accumulation of unique sets of proteins, RNAs, organelles, membranes, or cell-wall features. Stable cell polarity requires the breaking of symmetry and establishment of unique domains but also depends on the subsequent maintenance of this domain in the face of diffusion and membrane dynamics.

In this issue of *Developmental Cell*, Marhava et al. (2020) describe a mechanism by which a polar, membrane-associated protein module maintains itself through a biochemical feedforward loop. The two protophloem strands in the *Arabidopsis* root each connect the meristem to the remainder of the vasculature and consist of a single file of cells. Protophloem cell differentiation requires accumulation of the auxin signaling molecule, and a recent study by the same authors identified a molecular rheostat that maintains optimal auxin levels (Marhava et al., 2018). Auxin transport through protophloem cells is mediated by PIN-FORMED proteins, whose polar membrane localization directs intercellular flow (Wisniewska et al., 2006). PIN proteins are activated by the PAX protein kinase, which is in turn inhibited by the BRX protein. Auxin promotes BRX protein degradation, and this system therefore stabilizes auxin accumulation within a concentration range: when PIN proteins become too active, auxin levels decrease, causing BRX stabilization. BRX inhibits PAX, leading to reduced PIN activation and auxin accumulation. For this rheostat to operate, components need to be concentrated to the same membrane domain—but how?

A hint for a mechanism of membrane confinement came from the genetic analysis of the *brx* mutant. Mutations in the CVP2 phosphatase suppress the *brx* phloem phenotype (Rodriguez-Villalon et al., 2015). CVP2 catalyzes conversion of phosphatidylinositol-4,5-biphosphate (PIP2) to phosphatidylinositol-4-phosphate (PIP), and sensors for PIP and PIP2 show an enrichment of these phosphoinositides at the apical and basal membranes in root cells (Tejos et al., 2014; Marhava et al., 2018), consistent with them having roles in polarity. BRX localization depends on PAX, but not vice versa, and it was previously reported that PAX interacts with PIP2 (Barbosa et al., 2016). Mutating basic amino acids in PAX eliminates membrane association (Barbosa et al., 2016; Marhava et al., 2018) and renders the protein non-functional *in vivo*. Thus, PIP2 recruits PAX, which recruits BRX to polar sites. When identifying protein partners of PAX and BRX through immunoprecipitation, the authors surprisingly found PIP5K1 and PIP5K2 PIP2-generating enzymes. These proteins indeed localize polarly in protophloem cells (Tejos et al., 2014; Marhava et al., 2018), and this localization was mostly lost in *brx* or *pax* mutants. Thus, the PIP2-PAX-BRX module recruits PIP5K enzymes, which promote PIP2 synthesis, hence reinforcing polar localization of the module.

Closer inspection of the localization patterns of the interacting PAX, BRX, and PIP5K proteins revealed that all reside in a "muffin"-like domain at the center of the basal membrane in protophloem cells. In contrast, PIN1 protein occupied a "donut"-like domain surrounding the PAX/BRX/PIP5K area (Figure 1). The donut configuration was neither generic—other membrane-integral proteins did not show this—nor passive, because the initial arrangement in the new cell plate was continuous. Thus, the central donut hole is established after cell division, and this requires the PAX/BRX/PIP5K module, as mutants in each component caused PIN1 to localize to the muffin domain instead.

An intuitive explanation for the PIN donut would be enhanced endocytosis in the center, which is assumed for two reasons: first, PIN localization strongly depends on regulated endocytosis and exocytosis (Geldner et al., 2001), and second, PIP2 promotes Clathrin-dependent endocytosis (Ischebeck et al., 2013). The dynamics of a central endocytosis component—DRP1A—are consistent with the dynamics of the PIN1 donut. There is no DRP1A in the center of the cell plate, but after division, DRP1A co-localizes with the PAX/BRX/PIP5K muffin. What follows is a relatively simple feedforward mechanism: PAX binds to the membrane and recruits BRX and PIP5K. The latter synthesizes PIP2, which reinforces local PAX-membrane interaction. The increased PIP2 also locally promotes endocytosis of PIN1-containing vesicles (Figure 1).

The feedback mechanism is elegant and explains a number of so-far-disconnected phenomena. It provides a well-supported framework for understanding how polarity can be maintained at the basal membrane. At the same time, the study raises many new questions. Above all, one wonders if this is a unique mechanism utilized by protophloem cells. The aspect ratio of these cells is peculiar; they are very long cells with a minute apical/basal membrane surface. Here, this

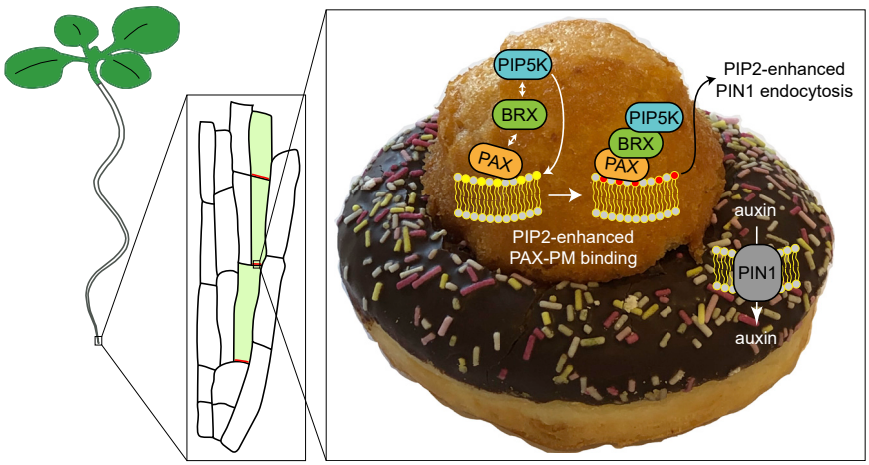


**Figure 1. Model of Feedforward Mechanism to Regulate Membrane Composition**
Within the basal membrane (red, middle panel) in *Arabidopsis* root protophloem cells (green cells, middle panel), a muffin-within-donut configuration (right panel) of PAX/BRX/PIPK5 (muffin) and PIN1 (donut) emerges from a feedforward loop in which PAX associates with the membrane and recruits BRX and PIP5K. PIP5K converts PIP (yellow balls in membrane) to PIP2 (red balls in membrane). This reinforces PAX-membrane association and enhances endocytosis of PIN1-containing vesicles, thus confining auxin transport activity to the surrounding membrane domain.

system can effectively regulate auxin flux by locally modulating endocytosis. Whether a similar module operates in cells with different geometries is an open question. It seems that at least the role of BRX in reinforcing cell polarity may be more widespread. BRX and its paralogs stabilize the polar localization of the BASL protein in the stomatal lineage (Rowe et al., 2019), a process that will likely involve a very different context of proteins and membrane domains.

Another key question is what kicks off the first polar targeting of PAX. A version that cannot bind PIP2 (Barbosa et al., 2016) still retains some polar membrane association (Marhava et al., 2018). Thus, initial PAX targeting seems independent of the later reinforcement mechanism. Does PIN protein polarization precede PAX polarization and provide an initial cue for its targeting? This is certainly possible, given the initially even cell plate localization of PIN1. It would be interesting to follow the dynamics of PIN1 and PAX localization during and immediately after protophloem cell division to establish an order of events. Following these proteins, as well as BRX, PIP5K, and PIP2 itself, would also help define how static or dynamic the complementary muffin and donut domains are. Are these stable landmarks, perhaps through reduced local membrane fluidity, or is there a high rate of lateral diffusion and a constant need for maintenance of the domains?

Finally, this study adds a very interesting dual role to the previously described rheostat. Not only does BRX inhibit PAX and PIN1 activation, it even stimulates removal of PIN1 from the membrane. It will be interesting to see if both modes of action occur within the same auxin concentration range or whether the enhanced endocytosis only kicks in once cellular auxin levels drop below a critical threshold.

## REFERENCES

Barbosa, I.C., Shikata, H., Zourelidou, M., Heilmann, M., Heilmann, I., and Schwechheimer, C. (2016). Phospholipid composition and a polybasic motif determine D6 PROTEIN KINASE polar association with the plasma membrane and tropic responses. Development *143*, 4687–4700.

Geldner, N., Friml, J., Stierhof, Y.D., Jürgens, G., and Palme, K. (2001). Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature *413*, 425–428.

Ischebeck, T., Werner, S., Krishnamoorthy, P., Lerche, J., Meijón, M., Stenzel, I., Löfke, C., Wiessner, T., Im, Y.J., Perera, I.Y., et al. (2013). Phosphatidylinositol 4,5-bisphosphate influences PIN polarization by controlling clathrin-mediated membrane trafficking in Arabidopsis. Plant Cell *25*, 4894–4911.

Marhava, P., Bassukas, A.E.L., Zourelidou, M., Kolb, M., Moret, B., Fastner, A., Schulze, W.X., Cattaneo, P., Hammes, U.Z., Schwechheimer, C., and Hardtke, C.S. (2018). A molecular rheostat adjusts auxin flux to promote root protophloem differentiation. Nature *558*, 297–300.

Marhava, P., Aliaga Fandino, A.C., Koh, S.W.H., Jelínkova, A., Kolb, M., Janacek, D.P., Breda, A., Cattaneo, P., Hammes, U.Z., Petrášek, J., et al. (2020). Dev. Cell *52*, this issue, 223–235.

Rodriguez-Villalon, A., Gujas, B., van Wijk, R., Munnik, T., and Hardtke, C.S. (2015). Primary root protophloem differentiation requires balanced phosphatidylinositol-4,5-biphosphate levels and systemically affects root branching. Development *142*, 1437–1446.

Rowe, M.H., Dong, J., Weimer, A.K., and Bergmann, D.C. (2019). A plant-specific polarity module establishes cell fate asymmetry in the Arabidopsis stomatal lineage. bioRxiv. https://doi.org/10.1101/614636.

St Johnston, D., and Ahringer, J. (2010). Cell polarity in eggs and epithelia: parallels and diversity. Cell *141*, 757–774.

Tejos, R., Sauer, M., Vanneste, S., Palacios-Gomez, M., Li, H., Heilmann, M., van Wijk, R., Vermeer, J.E., Heilmann, I., Munnik, T., and Friml, J. (2014). Bipolar plasma membrane distribution of Phosphoinositides and their requirement for auxin-mediated cell polarity and patterning in Arabidopsis. Plant Cell *26*, 2114–2128.

Wisniewska, J., Xu, J., Seifertová, D., Brewer, P.B., Ruzicka, K., Blilou, I., Rouquié, D., Benková, E., Scheres, B., and Friml, J. (2006). Polar PIN localization directs auxin flow in plants. Science *312*, 883.