IN BRIEF

Flipping the Vs: Integrating Vesicle Trafficking, PIN Polarity, and Plant Development

Plant cells need to know their tops from their bottoms. This is necessary to control basic aspects of development—where to place a new organ and which direction to grow in. It is also crucial for responding to the environment. Plants do this in a directional manner, growing stems toward intense light or roots downward in response to gravity and vice versa.

Plants use polarity cues to localize and relocalize members of the PIN-FORMED (PIN) family of proteins. PIN proteins export auxin and localize to one side of the cell (Wiśniewska et al., 2006). This is often coordinated across tissues, with PINs in many cells aligned in the same direction. This means that they can move auxin directionally and cause local developmental changes. A classic example of this is in the root: PIN2 is apically localized in root epidermal cells. After gravistimulation, apical PIN2 causes an auxin accumulation on the lower epidermis and makes roots grow downward (Wiśniewska et al., 2006). This is often coordinated across tissues, with PINs in many cells aligned in the same direction. This means that they can move auxin directionally and cause local developmental changes. A classic example of this is in the root: PIN2 is apically localized in root epidermal cells. After gravistimulation, apical PIN2 causes an auxin accumulation on the lower epidermis and makes roots grow downward (Wiśniewska et al., 2006).

In this issue of The Plant Cell, Zhang and colleagues (2020) show that the phospholipid flippase ALA3 (that shuffles lipids from one side of the membrane to the other) regulates PIN polarity. To show this, the authors ectopically expressed PIN1 under the control of a PIN2 promoter in pin2 mutant Arabidopsis (Arabidopsis thaliana) plants. These plants normally express PIN1 on the basal side of root epidermal cells. The authors identified an EMS mutant with apically localized PIN1 (see figure). They identified the causal gene as AMINOPHOSPHOLIPID ATPASE3 (ALA3) and showed that ala3 mutants exhibit an array of auxin-related developmental defects. These include increased root gravitropism, defects in root hairs, apical hook formation, venation patterning, and petal number, as well as enhanced auxin transcriptional responses during etiolation as measured by the auxin reporter DR5rev::GFP.

As predicted for a mutant with defects in membrane composition, ala3 mutants are defective in vesicle trafficking. They are more sensitive to brefeldin A, which inhibits ADP ribosylation factor guanine nucleotide exchange factor (ARF GEF) regulators of vesicle budding and causes membrane-localized proteins to be internalized as intracellular aggregates. Using the dye FM4-64, which marks lipid bilayers, Zhang and coworkers (2020) showed that ala3 mutants show increased endocytosis and disrupted trafficking to the Golgi and trans-Golgi network. To further test the role of ALA3 in ARF GEF-mediated vesicle trafficking, the authors crossed the ala3 mutant to the ARF GEF mutants big3 and gnom. They showed that big3 is epistatic to ala3, while ala3 and gnom act synergistically. They also used bimolecular fluorescence complementation and communoprecipitation assays in Nicotiana benthamiana to show that ALA3 interacts physically with GNOM and BIG3. These results suggest that ALA3 acts together with ARF GEFs to regulate PIN polarity via targeted vesicle trafficking.

These results echo findings in Caenorhabditis elegans and Saccharomyces cerevisiae, where ARF GEFs interact with flippases to regulate membrane properties. But many questions remain about how flippases link membrane properties to vesicle trafficking and subcellular polarity. One idea is that flippases generate an imbalance in phospholipids between the internal and external membrane surfaces, causing the membrane to bend inward and initiate vesicle budding (Lopez-Marques et al., 2014). Whatever future research holds, it will be fascinating to understand how different species use this poorly understood process to regulate fundamental aspects of development.

Chris Whitewoods
Department of Cell and Developmental Biology
John Innes Centre
Norwich, United Kingdom
christopher.whitewoods@jic.ac.uk
ORCID ID: 0000-0001-6886-3572

REFERENCES

Lopez-Marques, R.L., Theorin, L., Palmgren, M.G., and Pomorski, T.G. (2014). P4-ATPases: Lipid flippases in cell membranes. Pflugers Arch. 466: 1227–1240.

Wiśniewska, J., Xu, J., Seifertová, D., Brewer, P.B., Růžička, K., Bilou, I., Rouquié, D., Benková, E., Scheres, B., and Friml, J. (2006). Polar PIN localization directs auxin flow in plants. Science 312: 883.

Zhang, X., et al. (2020) Arabidopsis flippases cooperate with ARF GTPase exchange factors to regulate the trafficking and polarity of PIN auxin transporters. Plant Cell 32: 1644–1664.