Text by Ben Short
bshort@rockefeller.edu

$PI(3,5)P_2$ sows the seeds of plant growth

Phospholipid directs polarized growth by targeting actin-polymerizing formins to the cortex of plant cells.

In all eukaryotes, formin proteins nucleate and elongate actin filaments using conserved "formin homology" domains called FH1 and FH2. Many fungal and animal formins are regulated by Rho GTPases, but plant formins lack N-terminal Rho-binding domains (1). van Gisbergen et al. reveal that certain formins in the moss *Physcomitrella patens* are regulated instead by the phospholipid $PI(3,5)P_2$ (2).

Plant formins can be classified into two distinct groups (1), but analyzing the function of these proteins in vivo has proven difficult because many plant species express numerous members of each class. Mosses express fewer formins than other plants, however, a fact that, in 2009, allowed Magdalena Bezanilla and colleagues at the University of Massachusetts in Amherst to assign distinct functions to each of the two formin classes (3). "We showed that, in moss, class I formins are likely involved in cytokinesis," says Bezanilla, "whereas class II formins are essential for polarized growth."

This latter process involves the polarized secretion of new cell wall material at the tip of a chain of plant cells and the removal of excess membrane from the tip by endocytosis. Polarized growth is reduced in moss plants lacking the class II formins For2A and For2B and isn't restored by formin mutants lacking their C-terminal FH1 and FH2 domains (3). Yet the class II formins also require their N-terminal domains to promote polarized growth. "The N-terminal domain is responsible for the formins' apical localization," explains Bezanilla. "So we wanted to investigate what was driving this localization."

The N-terminal domain of class II plant formins is highly homologous to the lipid phosphatase PTEN (1, 4), though it lacks a key arginine residue required for lipid hydrolysis. Mosses express four different PTEN phosphatases, and the researchers, led by Peter van Gisbergen and Ming Li, found that fusing one of these homologues—PTENA—to the FH domains of For2A created a hybrid protein that could restore polarized growth to plants lacking class II formins (2). Fusing PTEND or human PTEN to the C terminus of For2A couldn't rescue formin-deficient mosses, however.

"The difference lies in their ability to bind $PI(3,5)P_2$," says Bezanilla, who found that PTENA and the N-terminal domain of For2A could bind to this phospholipid in solution, whereas PTEND and human PTEN couldn't. Moreover, fusing For2A's C terminus to $PI(3,5)P_2$-binding domains from completely unrelated proteins also created hybrids capable of rescuing polarized growth.

Surprisingly, however, none of these alternative $PI(3,5)P_2$-binding domains concentrated at the apical tip of moss cells. "So [For2A] doesn't need to be localized in the apical cytoplasm to be functional," Bezanilla says.

> "We also saw formin spots make new filaments as they moved along preexisting ones."

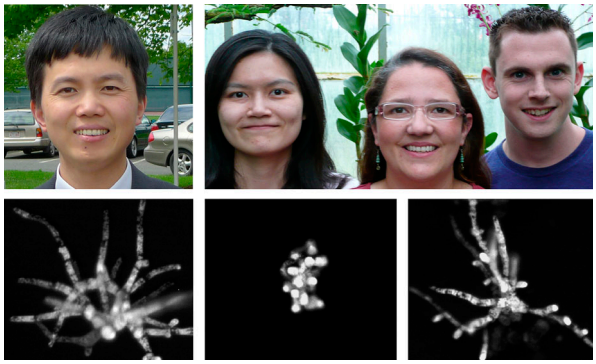
van Gisbergen et al. found that For2A also localizes to dynamic membrane patches around the cell cortex, and all the phospholipid-binding domains capable of functionally replacing For2A's N terminus showed a similar localization. "We think these spots are patches of membrane rich in $PI(3,5)P_2$," explains Bezanilla. Knocking down the lipid kinases that generate $PI(3,5)P_2$ in plant cells reduced For2A's cortical targeting and inhibited polarized plant growth.

Further supporting the idea that the cortical patches of For2A are functionally important, van Gisbergen et al. found that many of the spots were sites of active actin polymerization. "Fourteen percent of the spots move in linear trajectories, and this is actin dependent," says Bezanilla. "If we remove the actin with latrunculin, the cortical formin no longer moves [in straight lines]."

By simultaneously imaging For2A and actin, van Gisbergen et al. saw that the For2A patches generate new actin filaments as they move along linear trajectories. "They can do this in the absence of any other filaments, but we also saw formin spots make new filaments as they moved along preexisting ones," Bezanilla says.

The formin patches can therefore remodel cortical actin, though how this promotes polarized growth is unclear. "What's the connection to exocytosis and endocytosis?" Bezanilla asks, adding that she also wants to identify additional regulators that may switch formin on or off at the cell cortex.

Focal Point



PHOTOS COURTESY OF YING ZHOU (TOP LEFT) AND GRAHAM BURKART (GROUP PHOTO)

(Left to right) Ming Li, Shu-Zon Wu, Magdalena Bezanilla, and Peter van Gisbergen investigate how the activity of class II formins is regulated in order to promote the polarized growth of moss. The N-terminal domain of these formins is homologous to the lipid phosphatase PTEN, and the domain's ability to bind the phospholipid $PI(3,5)P_2$ is critical for targeting the formins to dynamic cortical membrane patches where actin polymerization is stimulated. Compared with a control plant (bottom left), a moss lacking class II formins is smaller and less polarized (bottom center). But polarized growth is restored by a hybrid protein containing the $PI(3,5)P_2$-binding domain of a human phosphatase and the actin-polymerizing domains of the moss formin For2A (bottom right).

1. Cvrckova, F., et al. 2004. *BMC Genomics*. 5:44.
2. van Gisbergen, P.A.C., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201112085
3. Vidali, L., et al. 2009. *Proc. Natl. Acad. Sci. USA*. 106:13341–13346.
4. Grunt, M., et al. 2008. *BMC Evol. Biol.* 8:115.