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News and Views

GAPs in ROP microdomains help establish cell polarity

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Rho GTPases are small proteins that act as molecular switches and are found in all eukaryotes, where they facilitate signaling to and from the plasma membrane (Boureux et al., 2007). Rho of Plants (ROPs) relay stimuli from outside and inside the cell to distinct plasma membrane domains where they influence processes like cell shape, tip growth, hormone responses, and more (Feiguelman et al., 2018). ROP circuits may create *de novo* cell polarity within the plasma membrane, laying the foundation to decide which part of the cell is "up" or "down" (Champneys et al., 2021). Interestingly, ROPs form distinct plasma membrane microdomains in plant epidermis pavement cells, which hints at some form of symmetry breaking and polarity establishment (Oda and Fukuda, 2012; Nagashima et al., 2018).

In this issue of *Plant Physiology*, Sternberg et al. have investigated the phenomenon of ROP microdomain formation in more detail. To understand this story, it is important to know how the ROP circuit works. The ROP activation and deactivation switch depend upon binding of GTP and GDP. In the GTP-bound state, ROPs are activated, while they are inactivated in the GDP-bound state. This cycling between active and inactive ROPs is regulated by two groups of proteins: ROP-Guanyl nucleotide Exchange Factors (ROP-GEFs) that dissociate GDP from ROPs and enable rebinding of GTP, and ROP-GTPase Activating Proteins (ROP-GAPs) that inactivate ROPs by enhancing hydrolysis of GTP (Feiguelman et al., 2018).

Earlier studies had shown spontaneous microdomain formation of ROP-GEF4 when co-expressed with ROP11 and ROP-GAP3; however, the presence of ROPs in these microdomains has not been clearly shown yet (Oda and Fukuda, 2012; Nagashima et al., 2018).

Sternberg et al. used transient expression of ROPs, ROP-GAPs, and a plant-specific ROP Nucleotide Exchanger (PRONE) domain of ROP-GEFs in *Nicotiana benthamiana* leaf epidermis pavement cells to study this microdomain formation. They found that a combination of various ROPs, together with ROP-GAP1 and ROP-GEF3^{PRONE}, resulted in microdomains of various shapes, either round, ring, or elongated (Figure 1). These microdomains themselves consisted of smaller nanodomains of about 1 μm and contained the ROPs almost perfectly colocalized with ROP-GEF3^{PRONE} but not GAP1. However, co-expression of GAP1 was necessary to induce microdomain formation. A constitutively active form of ROPs could form nanodomains together with ROP-GEF3^{PRONE}, but the higher-order microdomain structure was

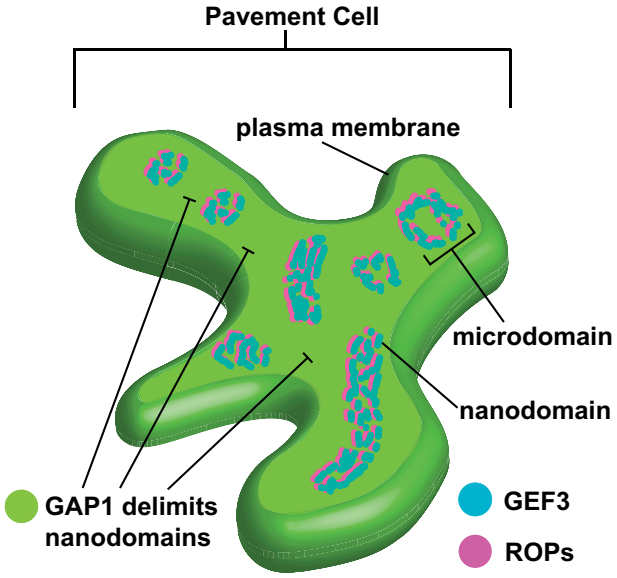

Figure 1 ROP microdomain formation in *N. benthamiana* pavement cells. Sternberg et al. co-expressed ROPs and regulators GEF3 and GAP1 in pavement cells. They imaged the upper plasma membrane of the cell and observed formation of small (1 μm) nanodomains that were structured into larger microdomains in which ROPs and GEF3 colocalized. GAP1 did not colocalize to these domains but played a crucial role in establishing microdomains by creating gaps in the distribution of the nanodomains.

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lost. When the constitutively active ROP was co-expressed with GAP1 and ROP-GEF3^{PRONE}, microdomain formation was not restored, suggesting that the deactivation of ROPs by GAP1 is important in microdomain formation by creating gaps in the distribution of nanodomains.

Time-lapse imaging showed that ROP microdomains were quite stable, so the authors sought to find what causes this stability. To study microdomain dynamics they employed a Fluorescence Recovery After Photobleaching beam-size analysis technique that can distinguish between fluorescence recovery by lateral diffusion or by membrane–cytoplasm exchange. With this technique, they showed that co-expression of ROP-GEF3^{PRONE} and GAP1 reduced the fluorescent recovery of ROP11 and that this was due to a restriction in lateral diffusion.

ROPs are categorized in either Group I or Group II based on their C-terminal domain and corresponding post-translation modifications. The ROP C-terminal domain facilitates interactions with phospholipids, which reside in the plasma membrane and can interact with proteins. The authors mutated ROP6 (Group I) and ROP11 (Group II) so that they could no longer bind these phospholipids. These ROP mutants were still able to form nanodomains but did not organize into microdomains, showing the interactions between special phospholipids and ROPs are crucial for microdomain formation and therefore polarity.

ROPs shape the cytoskeleton via interactions with actin and microtubule-binding proteins (Feiguelman et al., 2018). INTERACTOR OF CONSTITUTIVELY ACTIVE ROP 1 (ICR1) is an ROP interactor that also interacts with microtubules. Thus the authors tested if co-expressed ICR1 would also be drawn to ROP microdomains and how that would affect the microtubule network. Of Group I ROPs tested, only ROP6 recruited ICR1 to microdomains, while all Group II ROPS tested (9, 10, 11) recruited ICR1 to microdomains. However, in those cases, ICR1 still also located in microtubules, and the microtubules appeared to organize around the microdomain structures. This final finding showed the potential of ICR1 and Group II ROPs to organize the basic structure of the cell around microdomains and thereby change the organization of the cell.

To summarize, Sternberg et al. have discovered crucial details about ROP microdomain formation. They have shown that GEFs and GAPs are both needed but that they play different roles. GEFs help form the nanodomains, while GAPs are crucial to create the gaps in the nanodomain distribution, thereby helping from microdomains. This study has shed light on important fundamental building blocks that can determine the polarity of the cell. This is a crucial part of building a plant, or any form of multicellular life, and therefore deserves our full attention. The formation of polar domains in a cell determines where other determinants of polarity, such as the PIN auxin efflux carriers, preferentially locate to, which establishes the direction of growth and development of plants.

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