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# Connecting the plant cytoskeleton to the cell surface via the phosphoinositides

Camila Goldy<sup>1</sup> and Marie-Cécile Caillaud<sup>1\*</sup>

<sup>1</sup>Laboratoire Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRAe, F-69342 Lyon, France.

\*Corresponding author: [marie-cecile.caillaud@ens-lyon.fr](mailto:marie-cecile.caillaud@ens-lyon.fr)

## Abstract

Plants have developed fine-tuned cellular mechanisms to respond to a variety of intracellular and extracellular signals. These responses often necessitate the rearrangement of the plant cytoskeleton to modulate cell shape and/or to guide vesicle trafficking. At the cell periphery, both actin filaments and microtubules associate with the plasma membrane that acts as an integrator of the intrinsic and extrinsic environments. At this membrane, acidic phospholipids such as phosphatidic acid, and phosphoinositides contribute to the selection of peripheral proteins and thereby regulate the organization and dynamic of the actin and microtubules. After recognition of the importance of phosphatidic acid on cytoskeleton dynamics and rearrangement, it became apparent that the other lipids might play a specific role in shaping the cytoskeleton. This review focuses on the emerging role of the phosphatidylinositol 4,5-bisphosphate for the regulation of the peripheral cytoskeleton during cellular processes such as cytokinesis, polar growth, biotic and abiotic responses.

## Keywords

phosphoinositides, actin, microtubule, plant cell, cytoskeleton

## Introduction

The plant cytoskeleton plays essential roles in cell morphogenesis and is involved in the precise and dynamic organization of a plethora of processes, including growth, division, differentiation, and response to stress. The dynamics of the actin cytoskeleton drive membrane deformation and trafficking, which is required for polar cell growth and movement of vesicles and organelles. This dynamic

remodeling is controlled by diverse actin-binding proteins, which allow the nucleation of the actin filament (F-actin), its rearrangement, or dynamics<sup>1</sup>. At the same time, the microtubule cytoskeleton and microtubule-associated proteins are essential for cell division, anisotropic growth and mechanotransduction<sup>2,3</sup>. The continuum between the cell wall, the plasma membrane, and the cortical cytoskeleton allows the perception, integration, and propagation of both biochemical and biophysical signals<sup>4</sup>. In this context, membrane lipids present at the plasma membrane and facing the cytoplasm might act as a signaling platform in interaction with the underlying cytoskeletal network<sup>5</sup>. As such, the phosphoinositides, anionic glycerophospholipids derived from phosphatidyl inositol which can be phosphorylated, forming distinctive species of phosphatidylinositol-phosphate and bisphosphates<sup>6</sup> are good candidates. As essential determinants of the plasma membrane, the phosphatidyl inositol 4 phosphate (PI4P) and phosphatidyl inositol 4,5 bisphosphate (PI(4,5)P<sub>2</sub>) act in concert with phosphatidic acid and phosphatidylserine<sup>6,7</sup>. Our knowledge of the interaction between phosphatidylserine and the cytoskeleton is very limited, while evidence for the interaction between phosphatidic acid metabolism and the cytoskeleton is growing fast<sup>8–14</sup>. Here, we review the recent insights regarding the importance of PI(4,5)P<sub>2</sub>, known to be specifically enriched at the plasma membrane in the interaction between the cytoskeleton and the cell periphery. In particular, we focused on recent findings pointing toward a role of PI(4,5)P<sub>2</sub> patterning for the dynamics of cortical cytoskeletons, in the context of cytokinesis, polar growth, and stress responses.

## Cytokinesis

A plant-specific pathway heavily relying on polarized membrane trafficking is the establishment of the cell plate during cytokinesis. Although mutants defective in phosphoinositide metabolism present aberrant cell division phenotypes<sup>15–18</sup>, the exact roles of phosphoinositides in coordinating membrane traffic during cytokinesis are still poorly understood.

Concomitantly to the apparition of the actin cytoskeleton at the apicobasal part of dividing cells<sup>19</sup>, PI(4,5)P<sub>2</sub> is also polarly localized and excluded from the cell plate until late cytokinesis<sup>18,20–22</sup> (Figure 1). Based on this observation, it is tempting to speculate that the composition in phosphoinositides could mediate the F-actin organization and cell division orientation by stabilizing the cell plate parallel to the apical-basal orientation. Functional analysis of key enzyme-producing PI(4,5)P<sub>2</sub> needs to be performed in detail, to test if the local increase in PI(4,5)P<sub>2</sub> production influences the dynamics of the actin cytoskeleton and thereby orients cell division.

On the other hand, the exclusion of the PI(4,5)P<sub>2</sub> from the expanding cell plate<sup>20</sup> raises the question of how the cell plate will change its membrane identity to become the newly formed PI(4,5)P<sub>2</sub>-enriched plasma membrane. In a recent preprint, it emerges that during late cytokinesis, opposing polarity

domains are formed along the cell plate after its fusion with the plasma membrane<sup>23</sup>. In its maturing domain, the cell plate becomes enriched in PI(4,5)P<sub>2</sub>— probably through the diffusion of the PI(4,5)P<sub>2</sub> lipid or PI(4,5)P<sub>2</sub> producing enzymes (Figure 1). By contrast, PI(4,5)P<sub>2</sub> is actively depleted from the cell plate domain that is still expanding. This active exclusion is likely mediated by the putative PI(4,5)P<sub>2</sub> phosphatase SUPPRESSOR OF ACTIN 9 (SAC9)<sup>23</sup>. In this expanding cell plate domain, SAC9 colocalizes with the MICROTUBULE ASSOCIATED PROTEIN 65 (MAP65-3), a key regulator of the microtubules organization during cytokinesis<sup>24,25</sup> (Figure 1). In *sac9* mutant, the ectopic accumulation of PI(4,5)P<sub>2</sub> correlates with an altered MAP65-3 dynamic and the emergence of an additional microtubule structure branching out from the newly formed cell plate<sup>23</sup>.

MAP65-3 localizes to the very tip of the microtubules as it colocalizes also with membrane marker at the cell plate during cytokinesis. Furthermore, MAP65-3 remains localized at the newly formed plasma membrane even after mitotic microtubules are gone<sup>24</sup>. This particular location suggests that MAP65-3 might interact not only with microtubules but also with certain components of the cell plate membrane. This could also be true for other proteins orienting cell division, present at the interface between microtubules and the plasma membrane<sup>26</sup>. During plant cell division, specific protein complexes marking the future cell division plane are succeeding each other's at a specific cortical region, named the cortical division zone (CDZ, Figure 1). Two members of the PLECKSTRIN HOMOLOG Y GTPase ACTIVATING PROTEINS (PHGAPS) were shown to be localized at the cortical division zone and to regulate key components of the cell division orientation, including MAPs<sup>27</sup>. Member of the GAP family are known to inactivate the Rho-like GTPases of plants (ROPs-RACs, Figure 1), while this feature was not yet characterized in the context of cell division. Moreover, whether or not the anionic lipid binding domain present in PHGAPs is important for their localization at the future cell division plane remains unknown<sup>27</sup>. Yet, protein–lipid overlay assays recently showed that PHGAP2 (also named ROP-ENHANCER (REN) 3) binds to PI(4,5)P<sub>2</sub>, and phosphatidic acid<sup>28</sup>, putting a member of the PHGAP family at the heart of the membrane-cytoskeleton continuum for orientation of the division.

## **Polarized growth**

Cell polarity in plants is regulated through the action of ROPs, which control cytoskeleton organization leading to the shaping of the pavement epidermal cell, pollen tube and root hair<sup>29,30</sup>. The coregulation of ROP, phosphoinositides and the phosphoinositide metabolism is proposed to mediate the coordinated activation of ROP through modulation of their guanine-nucleotide exchange factors (GEFs) and GAPs (Box 1). Even though, ROPs already associate with the plasma membranes *via* anionic lipids interaction<sup>31</sup>, regulators and effectors of the ROPs might also interact with lipids, linking thereby the plasma membrane to the cytoskeleton. As such, it was recently shown that the alternation of microtubule-rich indentations and microtubule-poor lobes depends on PHGAP1/2<sup>28,32,33</sup>. There,

PHGAPs associate only with a subset of microtubules at the anticlinal face, independently of F-actin. The polar localization of PHGAPs coincides with microtubule bundles in indentation regions and relies on the PHGAP C-terminal domain as well as on microtubules<sup>32</sup> (Figure 2a). How PHGAPs might be regulated, and whether this affects their polar and microtubule-dependent localization should be addressed in the near future.

Polarized cell expansion is observed during the tip growth of pollen tubes (Figure 2b). The fine balance of apical secretion and vesicle recycling at specific zones of the cell are orchestrated in concert with actin dynamics<sup>34,35</sup>, while microtubules seem to not have a direct function in pollen tube morphology<sup>36</sup>. In the subapical region of pollen tube, a so called “actin fringe” composed of short transverse cables<sup>37</sup> is thought to mediate vesicle trafficking including exocytosis and endocytosis toward the tip<sup>38</sup>. A recent preprint showed that the actin fringe involves the plasma membrane-anchored immunophilin-like protein TWD1, presumably through its function as adaptor for myosin-dependent cargo trafficking<sup>39</sup>. Whether or not this mechanism requires a specific lipid patterning at the subapical region of pollen tube remains to be addressed.

Overexpression in the pollen tube of the canonical members of PI4P 5-kinases results in secretion defects and tip branching phenotypes<sup>40</sup> while overexpression of other members cause pollen tube tip swelling, proposed to be linked with F-actin dynamics<sup>41,42</sup>. The presence of at least two functional pools of PI(4,5)P<sub>2</sub> within the subapical plasma membrane of the pollen tube<sup>43</sup> may be defined by the respective PI4P 5-kinases mediating their biosynthesis. This model is supported by a recent study showing that PI(4,5)P<sub>2</sub> accumulation observed using biosensors occurs both in membrane nanodomains and in a diffuse distribution at the subapical plasma membrane of tobacco pollen tubes<sup>44</sup> (Figure 2b). Both PI4P 5-kinases AtPIP5K2 and its putative tobacco ortholog NtPIP5K2 are associated with plasma membrane nanodomains, whereas canonical NtPIP5K6 does not<sup>44</sup>. When targeted to nanodomains, active PI4P 5-kinase variants stabilized F-actin, suggesting a role of the PI(4,5)P<sub>2</sub> pool present in nanodomain for F-actin dynamics<sup>44</sup>. We cannot exclude that other lipid classes—in particular PA<sup>45</sup>—would be present in the observed nanodomain at the tip of the growing pollen tube and thereby might contribute to the cytoskeleton dynamic together with PI(4,5)P<sub>2</sub>.

Root hair growth is in many ways (including lipid signaling) similar to pollen tube growth<sup>6</sup>. As such, anisotropic expansion of root hairs is generally thought to be exclusively mediated by tip growth, (Figure 2c). However, cell wall expansion was also observed along the polar growth axis on the root hair shank<sup>47</sup>. De novo deposition and enzymatic remodeling observed in the shank suggest that the cytoskeleton is also mobilized to drive delivery of cell wall components away from the apical dome<sup>47</sup>. Whether or not anionic lipids are also involved in this process remains to be elucidated. But it might be time to reevaluate the role of PI(3,5)P<sub>2</sub>, produced by FORMATION OF APLOID AND BINUCLEATE CELLS 1 (FAB1) as they were both described to localize on the shank and not at the growing apex of

the root hair (Figure 2c). In plants with reduced *FABIA* and *FABIB* expression, root hair shank stiffness is altered triggering a wavy root hair phenotype. This phenotype is likely linked with the defective incorporation of the xylan polysaccharides in the secondary wall and impaired cortical microtubules organization<sup>48</sup>. This finding put PI(3,5)P<sub>2</sub> as a possible link between microtubule and cell wall modifications in the regulation of shank growth.

## Plant microbe interaction

Some microorganisms developed the ability to interact and to colonized specific plant cell types such as root hair. In a recent study, investigating symbiotic interaction, tip-polarized enrichment of the PI(4,5)P<sub>2</sub> at the bacterial infection thread was also observed, which spatially coincided with low enrichment of the PI4P (Figure 3a). Such difference was much reduced in *rhizobium-directed polar growth* (*rpg*) mutant plants, which are critical genetic determinants of bacterial infection<sup>49</sup>. This result suggests that PI(4,5)P<sub>2</sub> may contribute to local membrane polarization at the tip of the infection thread. When imaging *rpg-1* infected root hairs, the microtubule array was no longer bridging the tip and the nucleus was mis-positioned<sup>49</sup>. Together, this study hints that polarized PI(4,5)P<sub>2</sub> might directly or indirectly guide the dynamic reorganization of the microtubule cytoskeleton during biotrophic interaction.

This hypothesis is further supported by findings observed during the plant pathogen interactions in leaves, where PI(4,5)P<sub>2</sub> accumulates at the plant membrane surrounding the pathogens while PI4P did not<sup>50–52</sup> (Figure 3c). Pharmacological treatment revealed that the dynamic movement of PI(4,5)P<sub>2</sub> into the plant membrane surrounding *E. cichoracearum* occurred via an actin-dependent transport pathway<sup>52</sup>. There, the depletion of the plasma membrane PI(4,5)P<sub>2</sub> pool by knockout mutation of PIP5K1 and PIP5K2, prevents susceptible responses and disease development of host plants against *Colletotrichum* spp and *Magnaporthe oryzae*<sup>52,53</sup>.

## Ionic stress tolerance and ER-PM contact sites

Plants often have to cope with different environmental challenges, including the negative effects of salinity on growth and development. In this context, the role of phosphoinositide as regulators of salt stress is emerging<sup>54</sup>. It has been pointed out that an increase in PI4P/phosphoinositol ratio in the plasma membrane in *Arabidopsis* is crucial for maintaining ionic homeostasis to protect plants from unfavorable environmental conditions<sup>55</sup>. These findings can be correlated with previous reports where salt stress induces an increase of PI4P and PI(4,5)P<sub>2</sub> content in plants<sup>56–58</sup>. At the cellular level, environmental stressors promote mechanical changes that can be sensed by the cytoskeleton and induce alterations in

the cellular microdomains that physically connect the cortical endoplasmic reticulum and the plasma membrane, called ER-PM contact sites<sup>59</sup>. It has been proposed a dual model where abiotic stress induces the slow rearrangements of ER-PM contact sites-localized protein complexes with an accumulation of PI4P in a cytoskeleton-dependent mode, and another fast and cytoskeleton-independent response that is driven by an increase in PI(4,5)P<sub>2</sub><sup>60,61</sup>. We can note that while the ER movement requires both actin and microtubules in interphasic cells<sup>62</sup>, the biological relevance of ER morphogenesis and ER-PM contact site during cell division or polar growth is still unclear.

Determining the molecular player regulating such interface between the cytoskeleton, the endoplasmic reticulum and the plasma membrane seems challenging but might be one of the future directions to understand the fine tune regulation of the dialogue between membranes and the cytoskeleton.

### **Concluding remarks and future perspectives**

Mutations in phosphoinositide-processing enzymes often result in drastic developmental defects and cytoskeleton disorder<sup>15,16,63–66</sup>. In turn, mutants impaired in microtubule self-organization, present altered PI(4,5)P<sub>2</sub> patterning within the Arabidopsis shoot apical meristem<sup>67</sup>. These findings, together with the defects in cortical microtubule orientation observed after the inducible depletion of PI(4,5)P<sub>2</sub> from the plasma membrane<sup>68</sup> highlights the crosstalk between the PI(4,5)P<sub>2</sub> and the microtubules. Whether or not microtubules associated proteins interact directly with anionic lipids is still poorly documented, although the implication of the ROP signaling pathway seems undoubtedly crucial to control plasma membrane-cytoskeleton continuum during polar cell growth. Few examples of interaction between microtubule associated protein with phosphoinositides were reported *in vitro*<sup>28,69,70</sup>, while the specificity of interaction with a given population of lipid has not been addressed *in vivo*.

While examples of effectors linking the anionic lipid to the microtubules are rare, evidence points towards an interplay between the plant phosphoinositides metabolism and the actin cytoskeleton, as already well known in animal cells<sup>71</sup>. As such, inducible depletion of PI(4,5)P<sub>2</sub> from the plasma membrane in Arabidopsis prevents the F-actin dynamics<sup>68</sup> and PI(4,5)P<sub>2</sub> specifically inhibits the ACTIN RELATED PROTEIN 2/3 (Arp2/3) complex-mediated actin polymerization *in vitro*<sup>63</sup>. It is likely that cell type specific mechanism exist that connect phosphoinositides with cytoskeleton dynamics. In this context, the direct interaction between the cytoskeleton associated proteins and the phosphoinositides, in particular PI(4,5)P<sub>2</sub>, might vary depending on the biological process, the cell type, or even at subcellular level on particular polar domain at the periphery of the cell.

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## **Declaration of competing interest**

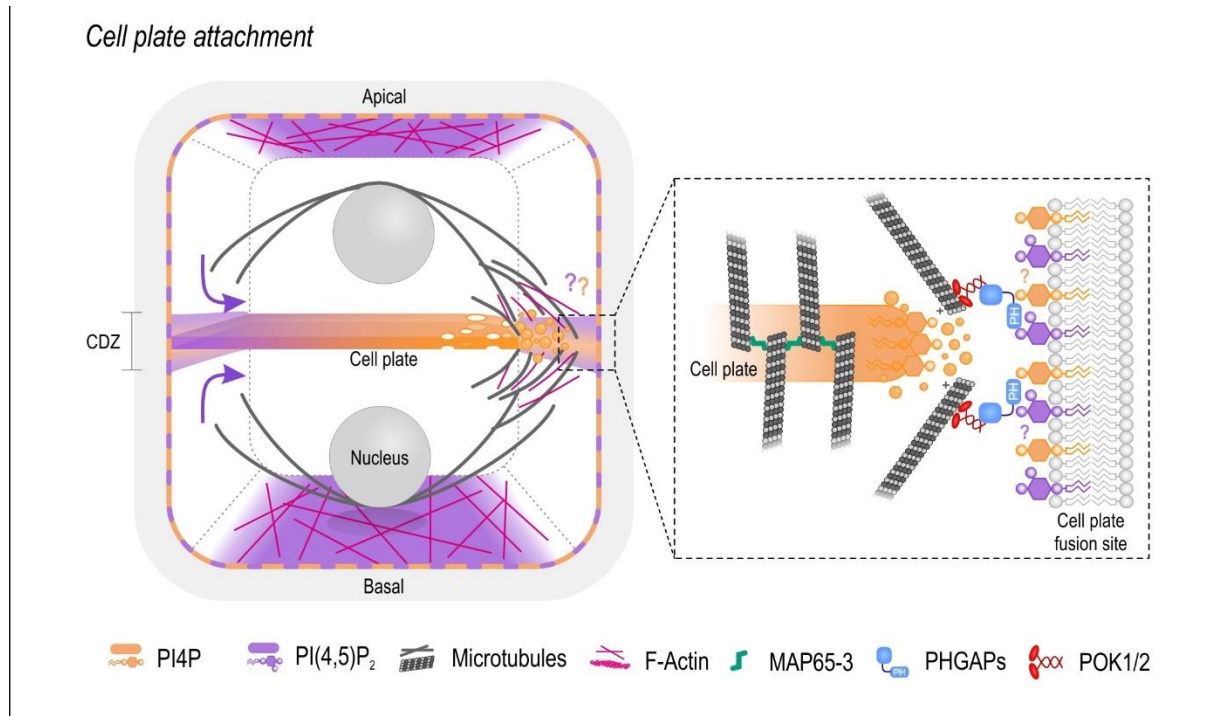
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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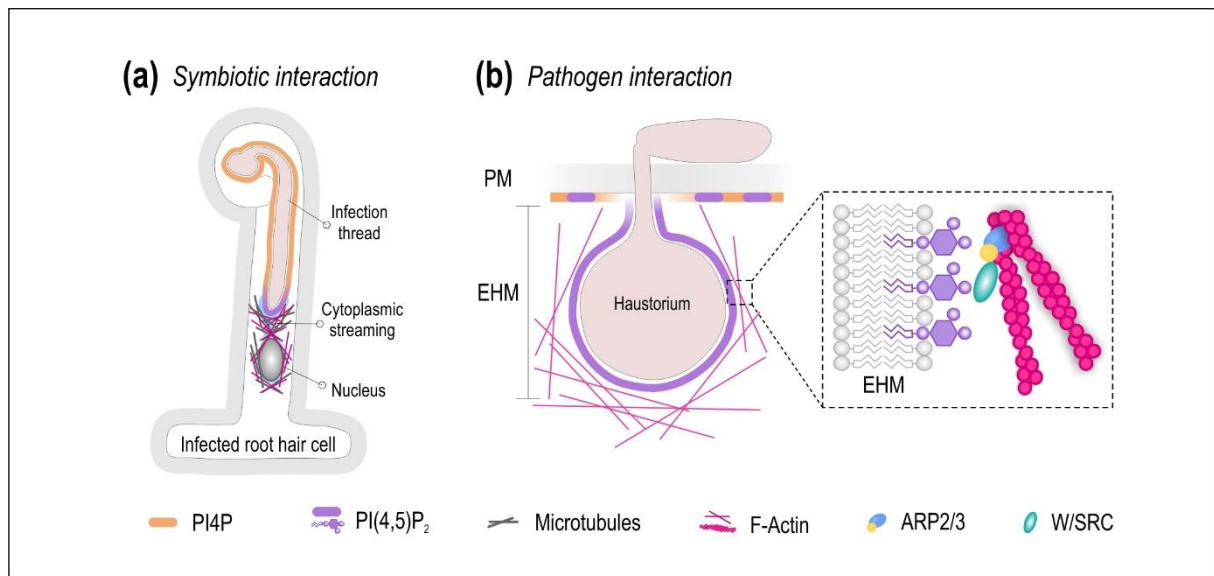


## Figures

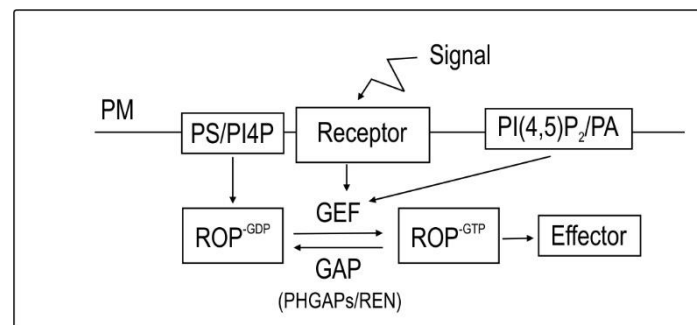


**Figure 1.** Schematic representation of a dividing cell in three dimensions during unilateral cell plate attachment. During cytokinesis, PI(4,5)P<sub>2</sub> and actin filaments are apicobasal accumulated<sup>1918,20–22</sup>. At the expanding cell plate (right side of the cell) PI(4,5)P<sub>2</sub> is excluded probably by the phosphoinositides phosphatase SAC9<sup>23</sup>. Maturing cell plate (left side) becomes enriched in PI(4,5)P<sub>2</sub> perhaps by lateral diffusion and phragmoplast's microtubules disappear<sup>23</sup>. The boxed area is enlarged to show the details of cortical division zone (CDZ), where PI4P and/or PI(4,5)P<sub>2</sub> might act as anchors of microtubules via PHGAPs and other CDZ proteins (POKs).





**Figure 3.** Distribution of PI(4,5)P<sub>2</sub> and cytoskeleton organization during plant-microbes interaction. (a) In symbiotic interaction, PI(4,5)P<sub>2</sub> is enriched at the tip of the infection thread and this polar localization might guide the reorganization of the microtubule cytoskeleton and nucleus migration<sup>49</sup>. (b) In pathogen infection, PI(4,5)P<sub>2</sub> is accumulated at the extra haustorium membrane (EHM) in an actin dependent way<sup>50</sup>. The boxed area is enlarged to show the details of the interplay among PI(4,5)P<sub>2</sub>, W/SRC (WAVE/SCAR regulatory complex) and ARP2/3 complex, which binds to F-actin to induces cytoskeleton assembly and branching<sup>63</sup>.



#### Box 1. Simplified molecular toolbox of ROP signaling.

The establishment of cell polarity is essential for many processes such as spatial patterning of cell division and polar cell growth. Rho-like GTPases from Plants (ROPs) play a key role acting as signaling hubs regulating cytoskeleton reorganization, vesicle trafficking, cell polarity and morphogenesis. ROPs cycle between an active GTP-bound and an inactive GDP-bound state. Guanosine nucleotide exchange factors (GEFs) induce the exchange of GDP for GTP to activate ROP-GTPases, while ROP inactivation is facilitated by GTPase-activating proteins (GAPs). ROP signaling is transmitted by various effectors, which preferentially interact with the active forms of ROP, but not the inactive forms. Polar distribution of ROPs might be established by electrostatic interactions with anionic lipids. In addition, some ROP regulators also interact with anionic lipids, like REN and PHGAPs. It is becoming clear that several membrane lipids regulate ROP localization in particular domains at the cells, however the mechanism behind this polar localization is still unknown.

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