CELL POLARITY

ARMADILLOs delimit Rho signalling

Polar cell growth requires spatiotemporal regulation of Rho of plants (ROPs) small G proteins in membrane domains. In addition to localized activation of membrane-anchored ROPs, a mechanism for their local inactivation has now been identified.

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Plant development and shape formation is dependent on the establishment and maintenance of polar cell growth. Central to the regulation of cell polarity are Rho of plants (ROP) small GTPase family. ROPs are membrane-anchored molecular switches, which exist in a GTP 'ON' or GDP 'OFF' state. They are activated by ROP guanine nucleotide exchange factors (GEFs), which facilitate GDP release and are rapidly inactivated by ROP GTPase-activating proteins (GAPs)[1]. Rho superfamily GTPases-based polarity is generally thought to take place by highly restricted GEF distribution and more widely dispersed GAP distribution, a mechanism often referred to as 'local activation and global inhibition'. In budding yeast, the Bem1 scaffold mediated restriction of Cdc42 and its GEF Cdc24 has been established as a paradigm for localized activation and polarity generation by Rho GTPases[2]. In plants, local accumulation of GEFs during root hairs and trichome development as well as in secondary cell wall pits formation has been associated with local ROP activation and regulation of cell polarity[3–5]. In this issue, Kulich et al. describe a novel scaffold-associated mechanism that locally restricts GAP distribution[6], highlighting the importance of ROP inactivation in regulation of cell polarity.

Two types of ROPGAPs have been identified in plants: the Cdc42- and Rac-interacting binding (CRIB) domain containing ROPGAPs and the pleckstrin homology (PH) domain-containing ROP1 enhancer (REN, also known as RENGAPs or PHGAPs). In pollen tubes, ROPGAPs localized to the shank[7], while the RENs localize to subapical cytoplasmic vesicles, restricting ROP activity to the tip[8].

Kulich et al.[6] also report that three *Arabidopsis* ARMADILLO REPEAT ONLY (ARO) proteins, ARO2/3/4, regulate root hair and trichome development by functioning as plasma membrane-associated scaffolds, which recruit RENs and GTP-bound ROPs to specific sites in the plasma membrane (Fig. 1). The AROs form an evolutionarily conserved family of proteins found in bryophytes and higher plants that contain two separate ARMADILLO (ARM) repeat motifs. Complementation assays with a *Marachantia polymorpha* ARO homologue suggest that ARO function is conserved and present an ancient mechanism for the regulation of ROP signalling in plants.

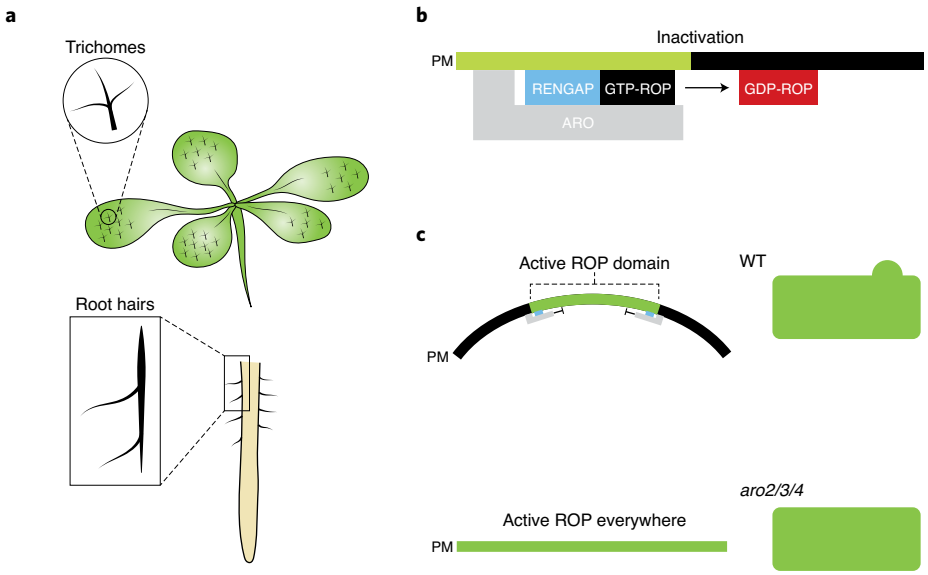

Fig. 1 | Spatial regulation of ROP function by AROs and RENs. a, ROPs regulate polar growth of root hairs and trichomes. **b**, AROs function as plasma membrane-associated scaffolds which bind both RENGAPs (also known as PHGAPs) and GTP-bound ROPs, leading to ROP inactivation. **c**, AROs associate with specific membrane domains, leading to delimitation of the active ROP domain (green region) and consequential polar growth. In *aro2/3/4* triple mutants, polar growth is lost due to expansion of the active ROP domain.

First, the expression pattern of *ARO2*, *ARO3* and *ARO4* was examined using a fluorescent reporter gene controlled by the promoter sequence of each of the three genes. This analysis showed that the AROs are expressed in bulging trichoblasts (root-hair-forming cells) and developing trichomes. Examination of transfer DNA (T-DNA) insertion mutants followed. While single mutants in each of the *ARO*s did not display a visible phenotype, the combined mutation in *aro2/3* and the triple *aro2/3/4* mutants developed irregularly shaped, split or burst root hairs. The *aro2/3/4* triple mutants also developed fewer fully matured trichomes and a large proportion of burst trichomes.

To obtain further insight into the function of AROs, their subcellular distribution was studied in developing trichomes, root hair and root epidermal cells using combination of confocal and total internal reflection fluorescence/variable angle epifluorescence microscopy (TIRF/VAEM) microscopies as well as biochemical fractionations. The combined analysis revealed that AROs are localized in plasma membrane-associated punctate structures at the apex of new trichome branches and apical membrane of root hair bulges. Membrane localization and complementation assays demonstrated that AROs interact with anionic lipids in the

plasma membrane through an N-terminal polybasic region (PBR) and that the interaction with the plasma membrane is required for their function.

The AROs interaction with RENs was identified by a yeast two-hybrid screen and was confirmed in vitro, as well as in vivo. Quantitative interaction assays by microscale thermophoresis (MST) revealed that AROs interact with different RENs with different affinities, suggesting that there could be differential regulation of the RENs by the AROs. An additional intriguing result showed that AROs interact with GTP-bound active ROPs, implying that AROs likely function as scaffolds which bring the RENs and the GTP-bound ROPs in proximity. Yet, MST measurements showed that AROs' interaction with RENs was two orders of magnitude stronger than with ROP1, suggesting a complex regulation.

Finally, the distribution of ROP2 and REN1 were compared between the wild type and *aro2/3/4* triple mutant. In *aro/2/3/4* trichoblasts, ROP2 membrane polarity was lost at the site of future bulge formation. In root hair bulges and trichomes of *aro2/3/4*, ROP2 localization was spread along the membrane, REN1 did not localize to the membrane, and the colocalization of ROP2 and REN1 in the lateral plasma membrane of root hair bulges was lost. Hence, the localization analysis confirmed the protein–protein interaction studies and demonstrated that the loss of polarity in root hairs and trichomes in the *aro2/3/4* triple mutant is associated with apolar ROP2 distribution, which likely results from compromised recruitment of RENs to specific plasma membrane domains.

Spatiotemporal regulation of ROP GTPase activation status has focused mostly on the manner in which ROP activation is spatially confined. Kulich et al.[6] describe for the first time a mechanism for spatial restriction of ROP inactivation by RENs and AROs. The identification of AROs as ancient and highly conserved scaffolds which regulate ROP signalling indicates that a spatially restricted inactivation of ROPs is as crucial as localized activation. AROs precisely constrain both unidirectionally tip-growing cells, such as root hairs and likely pollen tubes, and more complex diffuse growth of multipolar shaped cells, such as trichomes, through a unified cellular mechanism. AROs interact with both RENs and ROPs as well as vesicles and other interactors[6], suggesting that in addition to their importance in spatiotemporal regulation of ROP activity, they may also facilitate and integrate more complex signalling crosstalk. There are several critical open questions for future studies, such as: whether in pollen tubes the function of ARO is also associated with the regulation of ROP polarity; what is the regulation of ARO–REN–ROP complex formation and their dynamics; whether AROs also function in other cells in addition to root hairs, trichomes and pollen tubes; whether the function of RENs is also ARO independent; and whether AROs regulate ROP polarity in a similar fashion in bryophytes and other plant species. ❐

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Competing interests

The authors declare no competing interests.