Endocytic signaling in leaves and roots: same rules different players

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INTRODUCTION

The generation of planar cell polarity (PCP) is a process involving the distribution of cellular structures or molecules asymmetrically. PCP establishment requires a mechanism for the formation of both intra-cell polarity and inter-cell polarity.

Rho-like GTPases (ROPs) from plants are the sole signaling small GTPases in plants and it is therefore expected that they have a role in numerous signaling events. ROPs are already known to participate in signaling pathways that regulate cytoskeletal organization and vesicular trafficking, and as a consequence have an impact on cell polarization, polar growth, and cell morphogenesis. Microtubules (MTs) and actin microfilaments (F-actin) are the two major cytoskeletal elements that play a key role in many cellular processes, including cell polarity and endocytosis.

In plants, the phytohormone auxin has a cardinal role in the coordination of many physiological functions, including growth and the development of cells and organs (Benkova et al., 2003; Friml et al., 2003; Bilsou et al., 2003; Vlot et al., 2005; Wei et al., 2005; Wei et al., 2005; Scarpella et al., 2006; Wiesneki et al., 2006; Grimes et al., 2007; Gao et al., 2008; Yang, 2008). To function, auxin must be dynamic both spatially and temporally (Santner and Estelle, 2009; Vanneste and Friml, 2009). In multicellular plants, this process is in part mediated by the polar distribution of the auxin efflux carriers PIN-FOREM (PIN) proteins, which are required for polar auxin transport and the formation of auxin gradients.

Asymmetric endocytosis and the recycling of PINs localized at the plasma membrane (PM) contribute to the polar localization of PINs (Geldner et al., 2003; Dhonukshe et al., 2008). More recently, auxin has been implicated as a self-organizing signal that causes the polarization of PIN proteins. The auxin signal that appears to regulate downstream ROPs involved in PCP is mediated through auxin binding protein 1 (ABP1). ABP1 has been proposed to regulate clathrin-mediated endocytosis in roots, and the ROP-dependent pavement cell (PC) interdigitation in leaves (Robert et al., 2010; Xu et al., 2010, 2011).

The signaling mechanisms involved in the formation of cell polarity, including ROPs, their close relationship with the cytoskeleton and endocytic trafficking are the focus of this review. The above-mentioned mechanisms are all conserved in plants and animals, and consequently advances in knowledge in the plant system may synergize advances in understanding similar mechanisms and processes in mammalian systems.

SIGNALING AND ENDOCYTOSIS IN PAVEMENT CELLS

The formation of the jigsaw puzzle-like shape of Arabidopsis leaf PCs epitomizes a long-standing question in cell and developmental biology: How does a field of cells precisely coordinate uniform cell polarity? Importantly, the interdigitation of PCs provides an excellent system for the investigation because interdigitation is a non-essential process. It is therefore possible to study the signaling mechanism with the use of overexpressing or knockout plant lines.

In leaf PCs, the auxin cell surface receptor ABP1 mediates auxin signaling to coordinately activate two mutually exclusive ROP signaling pathways. They are activated in complementary lobe and indent regions on adjacent sides of the cell (Figure 1). A lobe in a cell corresponds to an indent in the adjacent cell. ROP2 and ROP4 promote lobe formation and are functionally redundant; ROP2 is
the dominant ROP in lobe promotion and it is common to refer to ROP2 and ROP4 simply as ROP2. ROP6 is responsible for the promotion of indentations (Fu et al., 2002, 2005). Both ROP2 and ROP6 localize to and are activated at the PM (Xu et al., 2010, 2011). The localization of the auxin efflux carrier PIN1 to the lobe tips requires localized ROP2, indicating the existence of a localized auxin–ROP2–PIN1–auxin positive feedback loop that could be responsible for the generation and maintenance of localized auxin levels (Xu et al., 2010). However, it remains to be established how auxin-activated ROP2 regulates PIN1 polarization. ROP2 regulates the formation of multipolarity through its activation of RIC4 (Fu et al., 2005), a member of the ROP interacting CRIB motif-containing (RIC) family of ROP effector proteins (Wu et al., 2001). RIC4 induces the formation of cortical F-actin in the tips of PCs (Fu et al., 2005).

In the indenting zone, ROP6 activates RIC1, leading to the formation of well-ordered MT arrays, which promote indentation and inhibit ROP2 activation (Fu et al., 2005, 2009). ROP2 inactivates RIC1, which causes the suppression of well-ordered cortical MTs, thus preventing outgrowth as MTs are excluded from outgrowing lobe tips (Fu et al., 2002, 2005). With the local activation of ROP2–PIN1 in the lobe region, ROP6 is suppressed at this site, given that the ROP2 and ROP6 pathways are mutually exclusive (Fu et al., 2009). Within an indent region, ROP6 localizes to and is activated at the PM (Xu et al., 2011). It is hypothesized that the mutual inhibition between the ROP2 and ROP6 pathways transforms the formation of well-ordered MT arrays, which promote indentation and inhibit ROP2 activation (Xu et al., 2005, 2009), leading to the establishment of the indenting zone (Xu et al., 2010). The mutual exclusivity of ROP2 and ROP6 helps restrict PIN1 to the lobe region.

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FIGURE 2 | A model in PCs for PIN1 polarization to the lobe regions of PCs via a ROP signaling mechanism. ROP2 is activated by extracellular auxin in the lobe region. The activated ROP2–RIC4 pathway leads to the inhibition of PIN1 internalization through RIC4-dependent cortical F-actin, leading to PIN1 polarization at the lobe. The PIN1-based export of auxin leads to further ROP2 activation for completion of this feed-forward cycle. Recent data (Nagawa et al., 2012) indicates that the activated ROP2–RIC4 pathway has a role in the promotion of endosomal trafficking from early endosomes to recycling endosomes (Brefeldin A inhibits ADP-ribosylation factor GEF and prevents endosomal recycling, the accumulation of internalized PIN1 in aggregates known as BFA bodies in plant cells). Endocytosed material can then be recycled back to the PM. Material maintained in early endosomes as they mature becomes internalized in late endosomes. The multivesicular structure of the late endosomes allows membrane fusion with the vacuole. Proteins within the late endosomes are delivered to the vacuole for degradation.

REGULATION OF ENDOCYTOSIS IN ROOTS

In roots, the mechanisms underlying apical and basal polarization appear similar to the coordination of polarity in leaves. In roots, recent findings have shown that a signal module composed of auxin, ABP1, ROP6/RIC1, clathrin, PIN1/PIN2 act as an integral component of the feedback regulation of auxin transport during root development.

Recent evidence indicates that ROP6 affects endocytosis and is involved in PIN internalization (Chen et al., 2012). Subsequent experiments revealed that the uptake of FM4-64 increased in the roots of rop6 or ric1 mutant plant lines, whereas uptake was reduced in the presence of constitutive rop6 expression (Chen et al., 2012). In addition, visualization of clathrin heavy chain with the aforementioned plant lines revealed that ROP6 signaling negatively regulates clathrin-mediated endocytosis (Chen et al., 2012). The role of the ROP6/RIC1 pathway in endocytosis is similar to the regulation of PIN interdigitation in leaves.
been begun to link auxin signaling to PIN-mediated pattern formation and morphogenesis in roots. A genetic screen found that the absence of SPIKE1 leads to increased lateral root density and retarded gravitropic responses matching the phenotype observed in pin2 knockouts (Lin et al., 2012). Mutant spkl plants induced PIN2 internalization that could not be suppressed by auxin, equivalent to rps7 and ric7 mutants. Moreover, SPIKE1 was required for auxin induction of ROP6 activation.

The current model for the polar distribution of PIN2 via the ROP-based signaling pathway is presented in Figure 3.

CONCLUSION

Recent findings suggest that PIN internalization by ROP-based auxin signaling is a mechanism responsible for the regulation of polar auxin trafficking in plants. The ROP2/RC4 pathway being responsible for the induction of F-actin in PCs which leads to the inhibition of PIN1 internalization necessary for PIN1 polarization to the lobe tips (Nagawa et al., 2012). The ROP6/RC1 pathway functions in roots to inhibit PIN2 internalization through the stabilization of F-actin. A key distinction is that ABP1 activates the ROP pathway in PCs (Xu et al., 2010; Nagawa et al., 2012), whereas in roots ABP1 is responsible for inactivation of the ROP pathway (Chen et al., 2012). Future studies will hopefully elucidate whether this finding is due to differences in auxin concentration required to activate the ROP pathways in different tissues.

Whilst recent advances strongly suggest that the ROP-based auxin signaling that regulates PIN internalization is a widespread mechanism for the modulation of auxin transport in plants, key questions remain. Including for example the involvement of the ABF1 pathway in cyanoskeleton dynamics and cell polarity. A resourceful use of biochemistry, forward and reverse genetics, and imaging are necessary to identify the remaining components to obtain a fuller understanding of the signaling events regulating endocytosis in plants.

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Figure 3 | A model in roots for PIN2 polar distribution via a ROP signaling mechanism. Data indicate that the auxin-mediated inhibition of polar PIN2 internalization is regulated by the SPK1–ROP6–RIC1 pathway. Auxin is proposed to activate the SPK1–ROP6–RIC1 pathway and inhibit PIN2 internalization. The localized inhibition of PIN2 internalization via ROP6 signaling causes PIN2 to be retained at the PM, which generates a positive feedback mechanism for maintaining polar PIN2 distribution to the PM.
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