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Cell Polarity Signaling in *Arabidopsis*

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Abstract

Cell polarization is intimately linked to plant development, growth, and responses to the environment. Major advances have been made in our understanding of the signaling pathways and networks that regulate cell polarity in plants owing to recent studies on several model systems, e.g., tip growth in pollen tubes, cell morphogenesis in the leaf epidermis, and polar localization of PINs. From these studies we have learned that plant cells use conserved mechanisms such as Rho family GTPases to integrate both plant-specific and conserved polarity cues and to coordinate the cytoskeleton dynamics/reorganization and vesicular trafficking required for polarity establishment and maintenance. This review focuses upon signaling mechanisms for cell polarity formation in *Arabidopsis*, with an emphasis on Rho GTPase signaling in polarized cell growth and how these mechanisms compare with those for cell polarity signaling in yeast and animal systems.

Keywords

ROP GTPase; Rho GTPase; cytoskeleton; exocytosis; actin dynamics; polarized cell growth

INTRODUCTION

Cell polarity, broadly defined as asymmetry within a cell, is a fundamental feature of cell function that is tied to developmental and environmental regulation. Polarity formation is initiated by a polarizing signal, which regulates polar distribution of signaling molecules and leads to polarity establishment and maintenance through the cytoskeleton and vesicular trafficking. In plants, cell polarity is fundamental to development, growth, and morphogenesis in every stage of the life cycle. Zygote polarization is required for the first asymmetric division to the embryo axis. Similarly, polarized cell division is essential for cell differentiation and organ initiation and morphogenesis. Polarized cell expansion generates cells with specific shapes that are associated with specialized functions, e.g., polarized and guided pollen tube growth for sperm delivery. Finally, polar localization of auxin-carrier proteins allows directional auxin flow and auxin-gradient formation, processes that are essential for organ and tissue formation and growth.

Given the fundamental importance of cell polarity and its diverse forms, a crucial question in our understanding of this phenomenon is whether there are general principles underlying the various forms of cell polarity in all eukaryotic systems. This question is of particular interest considering the many unique features of plant cells, one of which, the cell wall, is indispensable for polarity formation. Indeed, a paradigm of polarity control centered on Rho GTPase

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The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

signaling has emerged from investigation of such diverse systems as yeast, worm zygotes, mammalian epithelial cells, plant pollen tubes (PT), and leaf epidermal cells (Figure 1). An equally important question is how diverse systems utilize these general rules to build the respective forms of cell polarity within specific developmental and environmental contexts. The answers to these questions have enhanced our understanding of general principles for cell polarity control and of the molecular mechanisms linking fundamental cell polarity to specific growth and developmental processes in plants. In this review, several plant model systems are discussed to illustrate how polarity control mechanisms in plants compare and contrast with those in animal and fungal systems.

MODEL POLARITY SYSTEMS IN PLANTS

Our understanding of the mechanisms underlying plant cell polarity formation has come primarily from several model systems (Figure 2), which offer fascinating biology and are accessible to experimental manipulations. Although the zygote of brown algae was formerly a favorite system (Fowler & Quatrano 1997), it has fallen out of favor because it is not amenable to genetic and molecular studies. Along with the development of *Arabidopsis* into a model plant, the single-cell pollen or multicellular epidermal systems (Figure 2) from *Arabidopsis* have been the focus of the investigation of the molecular and cellular biological and genetic bases of cell polarity generation in plants.

Tip-Growing Cells

Polar growth due to localized vesicle targeting and exocytosis to the growth site is termed tip growth, which occurs in all eukaryotic kingdoms and is required for the generation of highly elongated tubular cells such as fungal hyphae, animal neurons, PT, and root hairs (Figure 2). Although the mechanisms for tip growth in yeast have been extensively studied, it is unclear whether they apply to other tip growth systems, especially those involving rapid growth to an extraordinary length (e.g., neuronal axons, PT, and fungal hyphae). Both PT and root hairs serve as excellent model systems for rapid tip growth. Because root hairs are nonessential, many morphological mutants and concerned genes have been identified. Transcriptome analysis and the candidate gene approach have also identified important genes regulating tip growth. The genes identified by these approaches have provided some important insights into the molecular mechanism of tip growth, including signaling mediated by calcium, reactive oxygen species (ROS), and small GTPases (Bohme et al. 2004;Carol & Dolan 2006;Carol et al. 2005;Foreman et al. 2003;Grierson et al. 1997;Jones et al. 2002,2006;Molendijk et al. 2001;Parker et al. 2000;Preuss et al. 2006;Schiefelbein & Somerville 1990;Song et al. 2006;Wen et al. 2005).

To deliver sperm to the ovule, the PT must extend rapidly (as fast as 1 cm h^{-1}) and directionally by navigating through many tissues. This directional growth is remarkably similar to neuronal guidance in animals (Kim et al. 2003, 2004; Lord & Russell 2002; Palanivelu & Preuss 2006, Palanivelu et al. 2003). Unlike most other cells from multicellular plants, which dedifferentiate and lose their native polarity upon in vitro culture, cultured pollen maintains its developmental status and polarity. In vitro tubes grow synchronously and uniformly. As the growth of PT is genetically controlled by the haploid genome and involves many pollen-specific genes, lethal mutations or pollen-specific transgenes can be maintained in heterozygous plants, facilitating genetic analysis of the essential genes involved in polarity control. These advantages, combined with the ease with which live imaging can be performed in PT, make PT one of the most exciting systems for polarity studies in higher organisms.

Studies of tip growth mechanisms in PT have focused on two interlinking mechanisms: the oscillating tip–focused cytosolic calcium gradient and tip-localized dynamic Rho signaling. Both of these target the actin cytoskeleton that feedback-regulates them, constituting an

intricate signaling network known as LENS (localization-enhancing network, self-sustaining) (Cole & Fowler 2006). Given that there are roles for Rho GTPase, actin dynamics, and probably calcium in tip growth in other systems, LENS may be a useful conceptual framework for understanding the signaling mechanisms underpinning tip growth in various systems.

Morphogenesis of Epidermal Cells

Unlike root hairs and PT, most plant cells expand by diffuse growth, whereby they diffusely or uniformly increase their cell surface while certain regions or positions of the cell differentially expand owing to differential construction or remodeling of localized regions of the cell wall. Therefore, the mechanisms for localized cell-wall construction or modification are the key to the polarity of diffuse growth. These mechanisms have been investigated using several epidermal cell types amenable to microscopic imaging analyses (Figure 2). At least two forms of cell polarity are important for the morphogenesis of epidermal cells: the apical-basal polarity formed perpendicularly to the surface and the planar polarity along the surface. In *Arabidopsis* leaves, polarized growth from epidermal cells forming a trichome with three branches is controlled by apical-basal polarity. Trichomes are nonessential cells, and their morphology is easily observed, providing an excellent model for genetic analysis of morphogenesis. A large collection of mutants with altered trichome shapes has been isolated, and the molecular analysis of these mutants has demonstrated a critical role for the ARP2/3 actin nucleation complex in the regulation of trichome morphogenesis (Hulskamp et al. 1994,Schellmann & Hulskamp 2005,Smith & Oppenheimer 2005,Szymanski 2005).

Planar cell polarity, which involves coordination between cells within the plane of a cell layer, is essential for development and morphogenesis in animals (e.g., in convergent extension, wherein cells become intercalated to change the shape of early embryos). In plants, planar cell polarity is also critical for the differentiation of certain epidermal cell types (e.g., guard cells) and for the morphogenesis of most epidermal cells, such as trichoblasts in roots and pavement cells (PC) in leaves. Trichoblasts undergo polar diffuse growth to produce a bulge, from which tip-growing root hair forms. Interestingly, the site of the bulge formation, which is invariably adjacent to the basal end of the cross wall of trichoblasts (Figure 2), is regulated by the small-molecule hormone auxin (Fischer et al. 2006,2007).

PC with a jigsaw-puzzle appearance represent an exciting system for the investigation of polarity involving cell-cell coordination (Fu et al. 2002, 2005; Smith 2003). The development of PC with interdigitating lobes and indentations requires intricate and dynamic polarity formation (Figures 2, 3*a*) (Fu et al. 2002, 2005). The precise fitting of lobes and indentations among neighboring cells suggests a need for cell-to-cell signaling to spatiotemporally coordinate lobe outgrowth from one cell with the complementary indentation in a neighboring cell. This process bears a striking similarity to animal planar cell polarity, in particular to convergent extension requiring cell-to-cell intercalation (Goto et al. 2005, Klein & Mlodzik 2005, McEwen & Peifer 2001, Price et al. 2006, Settleman 2005). Interestingly, the similarity occurs at the mechanistic level as well. Dynamic gradients of a polarizing signal provide a developmental mechanism that activates the intercalary growth in PC (T. Xu, M. Wen, Y. Fu, J. Friml, J. Chen, M. Wu, A. Jones & Z. Yang, unpublished data); similarly, a gradient of unknown signal is thought to activate planar cell polarity in animals (Klein & Mlodzik 2005). Furthermore, accumulating evidence suggests that Rho GTPase–mediated cytoskeletal reorganization is important for planar cell polarity in animals, as has been shown for PC (Goto et al. 2005, Klein & Mlodzik 2005, McEwen & Peifer 2001, Price et al. 2006, Settleman 2005). Therefore, the study of PC polarity formation may help to shed light onto the mechanistic principles of planar cell polarity in other systems.

Polar Localization of Auxin Transporters

Cell polarity is often expressed as polar distribution of molecules within the cell; these molecules are not necessarily associated with detectable morphological expression in cell polarity. A good example of this type of polarity is polar localization of auxin transporters in plants, including auxin efflux carriers PIN-FORMED proteins (PINs) and influx carrier AUX1 (see Kleine-Vehn & Friml 2008, in this volume). The function of auxin is associated with its flow, its distribution as a gradient, the maximum of its gradient, and/or its specific concentration within a tissue or cell (Grieneisen et al. 2007, Wisniewska et al. 2006). All these features of auxin distribution and dynamics are primarily regulated by the polar distribution of PINs, with the polar distribution of AUX1 playing a secondary role (Kleine-Vehn & Friml 2008). Different PINs exhibit distinct polar localization patterns within the same cell, and the polarity of a PIN within the same cell is controlled by developmental or environmental signals (Leyser 2005). For example, the apical-to-basal reversal of PIN7 polarity in the basal cell of the two-celled embryo is important for *Arabidopsis* embryo axis formation (Friml et al. 2003). Therefore, signaling between PINs and polarity signals is required for polar localization of PINs.

Asymmetric Cell Division

As in other multicellular organisms, asymmetric cell division, found in zygotes and various stem cells, generates two unequal daughter cells with distinct fates in plants. Prior to asymmetric division, polarity must be established to allow this unequal distribution of cellular molecules and structures. For instance, in zygotes of higher plants the apical end is enriched with the cytoplasm, whereas the basal end is primarily occupied by a large vacuole. The mechanism for zygote polarization in plants remains poorly understood owing to the difficulties in visualizing the zygote embedded deep in the ovary and in isolating the mutations affecting this process, because they are lethal to the embryo. However, recent genetic studies on the differentiation of guard cells and root stem cells are beginning to unravel regulatory factors, such as transcriptional factor and signaling molecules, that modulate asymmetric cell division in plants (Aida et al. 2004, Bergmann et al. 2004, Hara et al. 2007, Nadeau & Sack 2002, Pillitteri & Torii 2007, Shpak et al. 2005, Wang et al. 2007, Xu et al. 2006).

THE CYTOSKELETON AND VESICULAR TRAFFICKING IN POLARITY CONTROL

A central aspect of the cell polarity paradigm is the significance of the cytoskeleton and its associated vesicular trafficking: Both serve as an essential cellular linkage to molecular pathways by responding to the initial polarity signal and providing spatial information for feedback regulation of the polarity-signaling pathways (Figure 1). Increasing evidence indicates that this paradigm can be extended to the regulation of plant cell polarity formation, although the detailed mechanisms by which the cytoskeleton and vesicular trafficking participate in plant cell polarity formation may vary from those in other systems. As in fungi and animals, both actin microfilaments (F-actin) and microtubules (MT), as well as targeted exocytosis and endocytosis, have been implicated in cell polarity control in plants (Cole & Fowler 2006,Murphy et al. 2005,Smith & Oppenheimer 2005). Given the tight linkage of these cellular events to polarity formation and Rho GTPase signaling, this review includes a discussion of their roles in plant cell polarity. For broader and more detailed descriptions of these cellular events, however, readers are referred to several recent excellent reviews (Campanoni & Blatt 2007,Cole & Fowler 2006,Ehrhardt & Shaw 2006,Murphy et al. 2005,Samaj et al. 2006,Smith & Oppenheimer 2005).

Microtubules

MT act to induce cell polarity formation by targeting and/or locally activating signaling molecules at the polar site in yeast and animal cells (Basu & Chang 2007, Siegrist & Doe 2007). A common signaling molecule regulated by MT in these systems is a Rho family GTPase, which controls actin-based polarity formation (Basu & Chang 2007, Siegrist & Doe 2007). Signaling in the polar site also feedback-regulates MT by stabilizing them. In plants, MT seem to have multiple roles and modes of action in cell polarity, some of which are analogous to polarity control in yeast and animals. Highly ordered paralleled cortical MT form a preprophase band (PPB) that predicts the site and the orientation of cell division, which are critical for asymmetric cell division. The localization of the PPB is determined by an initial polarity signal, which induces the polarization of cells prior to PPB formation. In this sense, the PPB acts to process but not to induce polarity. However, the PPB in turn induces the polarity of cell division, i.e., the position and the orientation of the new cell plate. PPB MT and the associated kinesins POK1 and ROK2 recruit TAN1 to the PPB cortical site, which signals to place and orient the phragmoplast according to the PPB (Muller et al. 2006, Smith et al. 2001, Walker et al. 2007).

Cortical MT modulate the polarity of cell expansion in various plant cells, but their modes of action may vary from one cell type to another. MT induce branching of *Arabidopsis* trichomes with three to four branches. Stabilizing the MT increases the branch number of wild-type trichomes and induces branching of un-branched mutant trichomes, whereas MT disruption causes unbranched trichomes (Mathur & Chua 2000). MT may regulate the localization of the Golgi apparatus or the targeting of specific molecules to induce trichome branching (Lu et al. 2005).

In diffusely growing cells, well-ordered cortical MT are thought to determine the region of the expanding cell surface; e.g., they are associated with the sinuses but excluded from the expanding lobes of PC (Figure 3). Removal of cortical MT results in isotropic cell expansion, implying that cortical MT may also directly participate in the formation of cell polarity in plants. Ordered MT are believed to guide the deposition of cellulose microfibrils for the restriction of cell expansion (Ehrhardt & Shaw 2006). This mode of MT action is clearly unique to plant cells. In addition, cortical MT modulate the polarity of cell expansion in plants by regulating Rho GTPase signaling (Figure 3) (Fu et al. 2005). This type of MT action in plant cells follows the general theme of the MT induction of cell polarity formation (Basu & Chang 2007,Siegrist & Doe 2007).

Actin Microfilaments

F-actin generally participate in polarity establishment by affecting the biochemical, structural, and biophysical properties of the plasma membrane (PM) at the polar site. Cortical F-actin, which form as patches or dense meshwork by the ARP2/3 actin nucleation complex, directly control PM polarization by regulating both exocytosis and endocytosis in yeast and animals (Basu & Chang 2007, La Carbona et al. 2006, Moseley & Goode 2006). Through vesicular trafficking or direct transport, cortical F-actin target signaling molecules (e.g., Rho GTPases or their activators) to the polar site to promote their own polymerization, forming a positive feedback loop for robust polarity establishment (Charest & Firtel 2006). Cytoplasmic actin bundles, which originate from formin-dependent actin assembly, maintain cell polarity either by targeting post-Golgi vesicles in yeast or by generating the trailing edge in animals.

The picture of F-actin's role in regulating cell polarity in plants is emerging but far from clear. The role of the ARP2/3 complex is limited to shape control in trichomes and in PC (Smith & Oppenheimer 2005). In PC, ARP2/3 knockout mutations do not eliminate but only alter the localization of a lobe-associated actin meshwork that appears as diffuse cortical F-actin

(Djakovic et al. 2006, Li et al. 2003). The formation of these ARP2/3–independent diffuse F-actin requires a Rho GTPase–dependent pathway (Fu et al. 2002, 2005).

The ARP2/3–independent F-actin also control tip growth in PT and root hairs. F-actin in the PT extreme apex have been a subject of debate for decades owing mostly to technical difficulties in visualizing the highly dynamic F-actin. Green fluorescent protein (GFP)-tagged mouse talin was used to visualize highly dynamic F-actin at the tip, which rapidly alternate between the cortex of the apical dome and the cortex immediately adjacent to the extreme apex (Fu et al. 2001, Kost et al. 1998). Rapid-freezing methods confirmed abundant presence of the latter but infrequent occurrence of the former (Lovy-Wheeler et al. 2005), probably reflecting snapshots of the dynamic apical F-actin. The existence of the former, however, is clearly supported by the finding that it is activated by a Rho GTPase signaling pathway localized to the extreme tip of the PM (Fu et al. 2001, Gu et al. 2005, Hwang et al. 2005).

The Rho GTPase–mediated dynamics of the apical F-actin is required for the generation or maintenance of PT polarity (Fu et al. 2001, Gu et al. 2005). In PT, evidence suggests that the actin dynamics regulates tip-targeted exocytosis by coordinating vesicle accumulation and docking/fusion (Lee et al. 2008). Rho GTPase–dependent actin assembly is required for vesicle accumulation to the apex, whereas its disassembly is critical for vesicle docking and/or fusion. Apical F-actin have also been implicated in positive feedback activation of Rho GTPases at PT tips, as in yeast and animals (Hwang et al. 2005). Therefore, at least some roles of dynamic F-actin in polarity formation are conserved in plants at the mechanistic level, although it is not clear whether the apical F-actin regulate endocytosis as they do in yeast and animal cells.

Exocytosis

A role for polarized exocytosis in cell polarity formation has been established in various eukaryotic systems (Brennwald & Rossi 2007, France et al. 2006, Mehta et al. 2005, Wedlich-Soldner et al. 2004, Zajac et al. 2005). Polarized exocytosis allows for tip growth in root hairs and PT by supplying membrane and wall materials; for instance, loss-of-function mutations on genes encoding the SEC3 and SEC8 subunits of exocyst, which control polar docking of vesicles to the PM, block tip growth (Cole & Fowler 2006). Polar exocytosis also mediates the targeting of signaling molecules. In yeast, polar activation of signaling molecules such as Cdc42 mediates polar exocytosis, which in turn leads to the molecules' own polar targeting, generating a positive feedback loop for robust generation of cell polarity (Wedlich-Soldner et al. 2004, Zajac et al. 2005). As mentioned above, the dynamic tip F-actin appear to participate in the positive feedback activation of ROP1 (where ROP refers to Rho-related GTPase from plants) by regulating exocytosis in pollen tubes. Polar localization of ROP GTPases to the PM involves ADP-ribosylation factor (ARF) GTPase–mediated vesicular trafficking (Molendijk et al. 2001; Xu & Scheres 2005a,b).

Knocking out a Rab4Ab effector depolarizes root hair growth (Preuss et al. 2006). Rab4A GTPases are localized to endosomes/trans-Golgi network (TGN)/exocytic vesicles that have accumulated at the tips of root hairs and PT (de Graaf et al. 2005; Lee et al. 2008; Preuss et al. 2004, 2006). Rab4A belongs to a 29-member subfamily of Rab GTPases that are predicted to localize to TGN/post-Golgi vesicles. The unusually large size of this Rab subfamily is consistent with the notion that these Rabs may be involved in the targeting of functionally distinct vesicles (Vernoud et al. 2003, Zhang et al. 2007). Specific molecules carried on RabA4 vesicles may either mark the site of polarity for tip growth or be involved in limiting or maintaining polarity signaling to the tip. In PT, a ROP1-negative regulator required for growth polarity control appears to be carried on tip-localized vesicles (J. Hwang, V. Vernoud & Z. Yang, unpublished data). Exocytosis-mediated targeting of negative regulators of Rho family GTPases may be a common mechanism for restricting polarity signaling to the polar site, as

has been shown for mammalian RICH1 (a CDC42 GTPase–activating protein) in restricting CDC42 signaling to the tight junction (Wells et al. 2006).

Polar localization of PIN and AUX proteins is another example of exocytosis of specialized vesicles that carry specific molecules (Dharmasiri et al. 2006; Geldner et al. 2003; Jaillais et al. 2006, 2007; Kleine-Vehn et al. 2006; Steinmann et al. 1999). Several recent studies clearly demonstrate that different auxin-transporter proteins are targeted to the PM through distinct exocytic routes involving SNX1-dependent and -independent endosome sorting, which are subject to regulation by specific polarity signals (Kleine-Vehn & Friml 2008).

Endocytosis

Endocytosis to retrieve the PM and its associated signaling molecules is a common mechanism for the maintenance of cell polarity in eukaryotes (Engqvist-Goldstein & Drubin 2003, Murphy et al. 2005, Nishimura & Kaibuchi 2007, Pruyne & Bretscher 2000, Yu et al. 2007). In yeast, CDC42 regulates not only polarized exocytosis but also endocytosis in its control of polarity formation. Computational modeling predicts that polarized targeting of PM proteins, their lateral diffusion in the PM, and their internalization by endocytosis are sufficient to generate sustained cell polarity (Marco et al. 2007). Endocytosis has been well documented in plant cells (Dhonukshe et al. 2007, Murphy et al. 2005) and occurs at the tips of PT and root hairs (Lisboa et al. 2007, Monteiro et al. 2005, Ovecka et al. 2005). A recent study in plant cells demonstrated the conservation of clathrin-mediated endocytosis (Dhonukshe et al. 2007), for which phosphatidylinositol 4,5-bisphosphate (PIP2) and its conversion to phosphatidylinositol 4-bisphosphate (PI4P) are required. Both PIP2 and PI4P are localized to the PT-apical PM, and alteration of PIP2 and PI4P levels inhibits the internalization of the PM at PT tips, leading to growth depolarization (Y. Zhao, A. Yan & Z. Yang, unpublished data). Furthermore, expression of the constitutively active form of ROP induces growth depolarization and inhibits endocytosis (Bloch et al. 2005). Interestingly, a screen designed to identify chemicals that affect the polarity of pollen tube tip growth has led to the identification of endocitin, which impacts the endocytosis of specific PM-localized proteins (Roberts et al. 2008). These observations support a role for endocytosis in maintaining polarized cell growth in plants.

Moreover, a large body of evidence shows that PIN proteins are internalized to endosomes by constitutive endocytosis (Dhonukshe et al. 2007, Richter et al. 2007, Teh & Moore 2007). Interestingly, auxin increases PIN localization to the PM by inhibiting endocytosis, forming a positive feedback loop of auxin efflux–signaling PIN localization (Paciorek et al. 2005).

SIGNALING MECHANISMS BEHIND CELL POLARITY FORMATION

How polarizing signals impinge on cytoskeletal reorganization/dynamics and vesicular trafficking is a central concern in polarity studies. A common theme of polarity signaling in plants has emerged from recent studies of polarized cell growth: Rho GTPase signaling networks, composed of multiple coordinate pathways and feedback loops, provide a robust molecular linkage between cytoskeleton and vesicular trafficking and polarity formation. This signaling mechanism not only resonates with the cell polarity paradigm in yeast and animals but also helps to reveal how Rho GTPases can be used to generate cell polarity in diverse systems. Rho functions as a hub in signaling by integrating numerous signals and coordinating multiple downstream pathways (Nibau et al. 2006, Yang & Fu 2007). To control the plant-specific aspects of cell polarity, Rho has evolved to interact with plant-specific partners or variants of conserved regulators.

ROP GTPases and Partners: Plant Reinvention of Conserved Polarity Signaling

In contrast to the multiple subfamilies of the mammalian Rho family (e.g., Cdc42, Rac, Rho, etc.), all plant Rho-like GTPases fall into a single subfamily, ROP (Figure 1) (Brembu et al. 2006,Burridge & Wennerberg 2004,Vernoud et al. 2003,Yang 2002,Yang & Fu 2007,Yang & Watson 1993). Six (ROP1 to ROP6) of the 11 *Arabidopsis* ROPs are known to regulate cell polarity formation (Gu et al. 2004,Yang 2002). Two classes of negative regulators, guanine nucleotide dissociation inhibitors (RhoGDIs) and GTPase-activating proteins (RhoGAPs), are conserved in plants, but they possess unique regulatory functions such as the Cdc42/Rac–interactive binding (CRIB) motif in RhoGAPs (Brembu et al. 2006,Wu et al. 2000). Interestingly, the conventional RhoGEFs (guanine nucleotide exchange factors) with the Dbl-homology (DH) domain are absent from plants, and only a single homolog of DHR2–RhoGEF, SPK1, is present in *Arabidopsis* (Basu et al. 2008,Brembu et al. 2006,Meller et al. 2005,Qiu et al. 2002). However, a novel family of plant-specific RhoGEFs known as RopGEF has recently been identified (Berken et al. 2005,Gu et al. 2006). Several pollen-expressed RopGEFs interact physically and functionally with a pollen-expressed receptor-like kinase (RLK), which belongs to a superfamily of receptor ser/thr kinases in plants (Kaothien et al. 2005,Zhang & McCormick 2007). RopGEFs and RLKs may have coevolved to form a plant-specific signaling module to respond to plant-specific extracellular signals (Yang & Fu 2007).

Plants have reinvented a set of Rho effectors, even though their signaling targets (e.g., the cytoskeleton) are conserved. Plant-specific effectors include two classes of novel proteins: a family of ROP-interactive CRIB motif–containing proteins (RICs) and a family of interactor of constitutively active ROP 1 proteins (ICR1 and ICR1-like) (Lavy et al. 2007, Wu et al. 2001, Xu & Scheres 2005a). RIC3 and RIC4 regulate actin dynamics in a manner apparently independent of the ARP2/3 complex (Fu et al. 2005, Gu et al. 2006, Li et al. 2003). Thus, ROP's regulation of actin dynamics in plants may involve a novel mechanism. ICR1 interacts with SEC3, an exocyst component known to be a direct effector of yeast CDC42. ICR1 is required for polarized growth of PC, consistent with a role in the activation of exocyst; however, direct evidence for this role is lacking (Lavy et al. 2007). These variations from the central theme of Rho signaling in cell polarity provide the basis for Rho GTPase control of cell polarity with various functionalities in diverse cell types and organisms. To elaborate further upon this idea, several examples of ROP signaling in cell polarity are discussed in detail below.

ROP GTPase Signaling at the Tip: The Pollen Tube Model

A role for ROPs in cell polarity control in plants was first suggested by a study showing ROP localization to the apical PM of pollen tubes and the essential role of ROPs in tip growth (Lin et al. 1996, Lin & Yang 1997). These initial findings and subsequent studies of ROP signaling have provided us with a road map for addressing key questions about tip growth (Figure 4): (*a*) What defines the apical PM domain (i.e., tip growth domain) for tip growth? (*b*) How is the tip growth domain generated and maintained? (*c*) How does this domain signal to exocytic vesicles to control their targeting and exocytosis to the tip?

The dynamics of tip-localized ROP activity drives tip growth—Three redundant ROPs (ROP1, −3, and −5), referred to as ROP1 for simplicity, are required for pollen tip growth (Hwang & Yang 2006, Kost et al. 1999, Li et al. 1999). As a positive regulator of tip growth, ROP1 activity is regulated and is critical for the polarity of tip growth (Gu et al. 2003, Hwang & Yang 2006, Kost et al. 1999, Li et al. 1999; J. Hwang, G. Wu, C. Grierson & Z. Yang, unpublished data). Live imaging of active ROP1 reveals the PM distribution of active ROP1 as a dynamic apical cap in normal oscillating PT (Figure 4*a*). It rises to the maximum size just prior to the growth peak and gradually decreases to the minimum followed by growth rate decreases (Hwang et al. 2005). The maximum of the active ROP1 cap determines the tip growth

domain, whereas the dynamics of the apical ROP1 activity drives tip growth (J. Hwang, G. Wu, C. Grierson & Z. Yang, unpublished data).

The dynamics of the active ROP1 controls tip growth in part by regulating the dynamics of apical F-actin (Fu et al. 2001, Gu et al. 2005, Hwang et al. 2005). Two ROP1 downstream pathways check and balance to control actin dynamics; the RIC4 pathway promotes the assembly of the apical F-actin, and the RIC3-calcium pathway promotes the disassembly of the apical F-actin (Figure 4). The actin dynamics not only modulates tip-targeted exocytosis for tip growth but also is required for the dynamics of the apical ROP1 cap through feedback regulations (Hwang et al. 2005, Lee et al. 2008).

The generation of the apical cap—ROP1 is first activated at the center of the future apical cap, and then spreads laterally to form the cap until reaching the maximum size (Figure 4*a*) (J. Hwang, G. Wu, C. Grierson & Z. Yang, unpublished data). Expression of a constitutively active ROP1 mutant (CA-ROP1) induces dramatic lateral spreading of endogenous active ROP1, producing a stable and dramatically enlarged ROP1 cap that is suppressed by RopGAP1 (J. Hwang, G. Wu, C. Grierson & Z. Yang, unpublished data). Furthermore, knocking out a RhoGAP produces a CAROP1-like phenotype (J. Hwang, V. Vernoud & Z. Yang, unpublished data). Finally, the ROP1 or RIC4 overexpression–induced depolarization of ROP1 activity is suppressed by Latrunculin B, which promotes actin depolymerization, implying that F-actin, downstream targets of ROP1 signaling, positively impact ROP1 activation (Fu et al. 2001, Hwang et al. 2005). On the basis of these observations, it was hypothesized that the lateral spreading of ROP1 activation is mediated by positive feedback (J. Hwang, G. Wu, C. Grierson & Z. Yang, unpublished data). One positive feedback mechanism involves apical F-actin (Hwang et al. 2005), similar to the F-actin-mediated positive feedback activation of Cdc42 in yeast (Wedlich-Soldner et al. 2004). Another positive feedback mechanism may involve RopGEF1, an ROP1 activator localized to the apical cap of PT, which was proposed to be directly activated by active ROP1 (Gu et al. 2006). In animal cells, a key feedback loop is Cdc2/Rac activation of phosphoinositide-3-kinase-mediated accumulation of phosphatidylinositol 3,4,5-triphosphate (PIP3) in the PM, which recruits Cdc42/Rac activators (Charest & Firtel 2006). Although there is no evidence for the presence of PIP3 in plants, roles of PI4P and PIP2 in polarized tip growth in root hairs and PTs have been demonstrated and have been implicated in ROP1 activation in PT (Dowd et al. 2006, Helling et al. 2006, Preuss et al. 2006, Stenzel et al. 2008; Y. Zhao, Y. An & Z. Yang, unpublished data). It would be interesting to determine whether these phosphoinositides participate in the positive feedback regulation of the apical ROP1 activity.

The maintenance and the dynamics of the apical cap through downregulation of ROP1—In wild-type PT, as soon as the apical cap of active ROP1 reaches the maximum, it needs to be reduced to confine the ROP1 activity to the tip growth domain and to maintain the dynamics of ROP1 activity (Figure 4*a*). The downregulation of ROP signaling may be mediated by either global and lateral inhibitions, or both. It has been proposed that the tobacco NtRopGAP1 acts laterally to restrict ROP activity to the apex on the basis of its subapical localization (Klahre & Kost 2006). Knocking out what appears to be the *Arabidopsis* ortholog of NtRopGAP1, however, does not cause the spreading of ROP1 to the subapical region, suggesting that this RopGAP does not play a major role in the downregulation of the apical ROP (J. Hwang, G. Wu, C. Grierson & Z. Yang, unpublished data). However, dramatic lateral spreading of apical ROPs and growth depolarization were induced by knocking out a pleckstrin-homology (PH) domain–containing RhoGAP, REN1 (J. Hwang, V. Vernoud & Z. Yang, unpublished data). REN1 protein is localized to the tube apex, as expected for a global inhibitor. Therefore, REN1 is the major RhoGAP globally inactivating the apical active ROP1.

Another global inhibitor of ROP1 is RhoGDI, which sequesters Rho in the cytosol to prevent it from being activated by the RhoGEFs. RhoGDIs are localized in the PT cytosol, as expected for a global inhibitor (Klahre et al. 2006). Knocking out RhoGDI2a in *Arabidopsis* induces tip swelling and lateral spreading of ROP1 from the tip, indicating that it is an important global inhibitor of the apical ROP1 (J. Hwang, G. Wu, C. Grierson & Z. Yang, unpublished data). A RhoGDI1 knockout also induces ectopic ROP2 localization to the PM, causing growth depolarization in root hairs (Carol et al. 2005).

Apart from global inhibitions, lateral inhibition may also play a role in limiting ROP1 activity to the tip. Cortical MT localized to the subapical region appears to have a minor role in restricting ROP activation to the apex. Stabilization of MT partially suppressed ROP1 overexpression–induced depolarization, whereas MT removal moderately increased lateral spreading of the apical ROP1 activity and growth depolarization (M. Zheng & Z. Yang, unpublished data). Both petunia phospholipase C (PLC1) and tobacco PLC3 are preferentially localized to the subapical region of the PM, and their inhibition induces growth depolarization (Dowd et al. 2006, Helling et al. 2006). Treatments with PLC inhibitors induced lateral spreading of active ROP1 (A. Yan & Z. Yang, unpublished data). These results support a role for PLC in limiting PIP2 and ROP1 activity to the tip.

Calcium: a critical link to feedback loops—As a self-organizing system, the downregulation of the apical ROP1 activity in PT is presumably activated by negative feedback mechanisms. Mathematical modeling predicts that RIC3-dependent calcium, which exhibits as oscillatory tip-high gradients (Holdaway-Clarke et al. 1997, Pierson et al. 1994), is a critical activator of the negative feedback regulation; this has been experimentally validated (A. Yan, G. Xu & Z. Yang, unpublished data). The nature of calcium-signaling targets in the negative feedback regulation is unclear, but mathematical modeling predicts that these targets may be either RhoGAP or an F-actin–disassembling factor, or both (A. Yan, G. Xu & Z. Yang, unpublished data).

Interestingly, calcium also participates in positive feedback regulation of ROS production, which is required for tip growth in root hairs. This growth in turn is catalyzed by the tip-localized RHD2 NADPH oxidase (Takeda et al. 2008). RHD2 appears to act directly downstream of ROP2 in root hair tip growth (Carol & Dolan 2006; Jones et al. 2002, 2007; Molendijk et al. 2001; Wong et al. 2007), and RHD2-mediated ROS activate influxes of tip-localized calcium (Carol & Dolan 2006, Jones et al. 2007), which in turn activates RHD2 to form a positive feedback loop consisting of RHD2, ROS, and calcium (Takeda et al. 2008). A similar ROS-calcium circuit may also regulate tip growth in PT (McInnis et al. 2006, Potocky et al. 2007). Given a role for the RHD2-calcium positive feedback loop in PT tip growth, how could calcium-mediated positive and negative feedback loops coexist in the same system? A simple model is that ROP activation of NADPH oxidase and RIC3 initiates the ROS-calcium positive feedback loop, which then runs independently of ROPs. This causes a calcium burst, which activates a negative feedback loop to downregulate ROPs.

Identification of calcium sensors will be necessary to test this model. A putative calcium sensor involved in feedback loops is calcium-dependent protein kinase (CDPK). Overexpression of a PM-localized petunia PiCDPK1 induced PT growth depolarization and excessive calcium accumulation (Yoon et al. 2006). Interestingly, a dominant negative mutant of PiCDPK1 also caused growth depolarization, whereas a constitutively active form of PiCDPK1 either blocked pollen germination or severely inhibited growth by reducing both the length and the width of PT. This CDPK may be the calcium sensor involved in both feedback loops.

Identifying ROP signaling–mediated calcium channels may also provide insights into feedback loops. A pollen-expressed putative calcium channel, CNGC18, is required for polarized PT

growth (Frietsch et al. 2007). CNGC18 is localized to the apical and subapical regions of PT PM and is permeable to calcium (Chang et al. 2007, Frietsch et al. 2007). ROP signaling enhances CNGC targeting to the PM, most likely through ROP-mediated polarized exocytosis, but it does not directly regulate its activity (Chang et al. 2007).

A model for tip growth signaling in pollen tubes and beyond—As discussed above, a model for tip growth signaling centered on ROP1 GTPase has been developed (Figure 4). This model may provide a framework for the understanding of detailed signaling mechanisms for various tip growth systems, e.g., growth of root hairs, fungal hyphae, and neurons. In root hairs, ROP2 probably activates tip growth through F-actin and calcium (Jones et al. 2002,Molendijk et al. 2001). RhoGDI1 plays a similar role in root hair tip growth as does RhoGDI2a in PT (Carol et al. 2005; J. Hwang, G. Wu, C. Grierson & Z. Yang, unpublished data). Rho family GTPases, actin dynamics, positive feedback loops, and RhoGAP-dependent global inhibition are also important for tip growth in other systems (Alberts et al. 2006;Bidlingmaier & Snyder 2004;Knaus et al. 2007;Ushinsky et al. 2002;Wendland & Philippsen 2000,2001). It will be interesting to see whether the interlinking feedback loops underlying the dynamics of the apical Rho GTPase cap in pollen represent a general mechanism for the control of tip growth in all systems.

Signaling between ROPs and Microtubules in Polarized Diffuse Growth: The Pavement Cell Model

The striking polarity of PC expansion makes it an ideal system for investigating how polarity is coordinated between cells and is controlled during diffuse growth, which is tightly linked to plant development and morphogenesis. In contrast to tip growth, cortical MT play a predominant role in determining the polarity of diffuse growth. Interestingly, current evidence indicates that polarity of diffuse growth is modulated by signaling mechanisms similar to those for tip growth. This similarity was first suggested by the observation that CA-ROP2 and DN-ROP2 mutants dramatically alter cell shapes in PC (Fu et al. 2002).

The ROP2/4–RIC4 pathway promotes lobe formation—Subsequent studies have shown that functionally redundant ROP2 and ROP4 promote lobe outgrowth (Fu et al. 2005). ROP2/4 are activated locally at the tips of growing lobes, where they promote localized accumulation of RIC4-dependent cortical fine F-actin, similar to the regulation of F-actin by ROP1 in PT tips. In addition, the lobe outgrowth may also require localized exocytosis. ICR1 was recently identified as a putative ROP effector that may link to exocyst recruitment to a polar site (Lavy et al. 2007). A ICR1 knockout generates small PC lacking lobes and indentations. Further studies should determine whether ICR1 is a ROP2/4 effector involved in recruiting exocysts. Active ROP2/4 also need to suppress the organization of ordered cortical MT in the region of outgrowth because ordered MT prevent outgrowth in diffusely growing cells (Figure 3) (Fu et al. 2002, 2005). Similarly, MT are also excluded from the tip growth domain in tip-growing cells, and it would be interesting to see whether active ROPs also play a role in excluding MT from the tip.

The signaling pathway promoting indentation—In the indenting region, RIC1 (a novel MT-associated protein) promotes MT ordering that restricts outgrowth, causing narrow necks (Figure 3) (Fu et al. 2005). Mutations in the CRIB motif of RIC1 eliminate the function of RIC1 in promoting MT ordering, implying that RIC1 may also be activated by a ROP GTPase to promote the organization of cortical MT (Y. Fu, Z.-L. Zheng & Z. Yang, unpublished data). These results seem to suggest a new ROP-RIC-mediated signaling pathway that activates MT ordering in plants (Fu et al. 2005). An increase in radial expansion of hypocotyl cells in the *ric1* null mutant suggests that this pathway apparently also restricts radial expansion of elongating cells with simple diffuse growth (Y. Fu, Z.-L. Zheng & Z. Yang, unpublished data).

Therefore, knowing which signals regulate this RIC1 signaling pathway may provide important insights into plant morphogenesis and development in general.

Microtubule suppression of outgrowth-promoting ROP signaling: a new paradigm for cell morphogenesis?—Cortical MT in the indenting region are known to signal back to ROP2/4 in such a way that ROP2/4 activation is repressed by cortical MT in the indenting region (Fu et al. 2005). In tip-growing PT, cortical MT present in the subapical region may also suppress ROP1 activation, probably by lateral inhibition (M. Zheng & Z. Yang, unpublished data). In root hairs, CA-ROP2-induced growth depolarization is enhanced by mutations in a kinesin that affects MT organization (Yang et al. 2007). Therefore, MT-mediated lateral inhibition of tip-localized ROP activation is involved in the regulation of tip growth polarity but is only secondary to the global inhibition. In contrast, cortical MT play a primary role in determining the polarity of diffuse growth, at least in part by suppressing outgrowth-promoting ROPs. This role can be extended to cells with simple diffuse growth, such as differentiating hypocotyls and root cells, in which cortical MT–mediated cell expansion in the direction perpendicular to MT orientation is primary, whereas ROP- and actin-induced cell expansion is secondary or minimal (Fu et al. 2002). On the basis of these observations, I propose that the interregulation between ROP signaling to actin-based polar growth and the polarity determination of cell expansion by cortical MT represents a unifying mechanism governing the polarity of cell expansion in plants, although the degree of significance for each of the two modes varies among tip growth and complex and simple diffuse growth (Figure 5).

Auxin: a small molecule acting as a common polarity signal?—The initiation of intercalary growth in *Arabidopsis* PC is tightly regulated in time and space during leaf development, as growth starts from the tip and progressively moves along the margin and finally toward the middle of leaves/cotyledons. Auxin forms dynamic gradients that are tightly correlated with the spatiotemporal progression of the intercalary growth, suggesting that auxin may be the polarizing signal that initiates the intercalary growth of PC (T. Xu, M. Wen, Y. Fu, J. Friml, J. Chen, M. Wu, A. Jones & Z. Yang, unpublished data). Auxin has also been implicated in the control of the positioning of root hair–forming sites, which appear to depend on ROP2 localization to those sites (Fischer et al. 2006). Therefore, an auxin-ROP2 pathway may also regulate the planar polarity of root hair positioning. Auxin is implicated in the regulation of the polarity of PIN localization, which is also planar in nature (Paciorek et al. 2005). This raises an important question: Is auxin-ROP signaling a common mechanism for cell polarity control in plants?

Given a role for auxin as a polarizing signal, an exciting question is how auxin, a diffusible small molecule, locally controls signaling to establish cell polarity. The answer may lie in polar localization of the auxin efflux carrier proteins, PINs. PIN-mediated/localized auxin efflux may lead to a localized accumulation of extracellular auxin, which in turn may locally activate ROP signaling to establish polarity. Auxin promotes PIN localization to the site of auxin accumulation in root cells (Paciorek et al. 2005). Thus, it is tempting to speculate that auxin may activate ROP2, which may promote polar localization of an auxin efflux carrier to the lobe tip to form a positive feedback loop (Figure 3). In this speculative model, the auxin receptor responsible for ROP2 activation would most likely be an extracellular receptor, which may directly regulate the potential ROP2 activator, SPK1, a RhoGEF whose knockout produces a PC phenotype similar to that of a *rop2/rop4* mutant (Basu et al. 2008, Fu et al. 2005, Qiu et al. 2002). PIN-exported auxin, which can diffuse across the cell wall to the indenting region of the neighboring cells, may also activate the ROP-RIC1 pathway as a possible mechanism for coordination between lobe outgrowth and indentation formation (Figure 3).

Polar Localization of Proteins: The PIN-FORMED Protein Model

Endosomal recycling and sorting are critical for polar localization and repolarization of PINs (see Kleine-Vehn & Friml 2008). How a recycled PIN decides to localize to a particular domain of the PM must require a signaling mechanism that integrates the specification of the PM domain with endosomal trafficking. Indeed, the PINOID (PID) ser/thr protein kinase and its antagonist, PP2AA phosphatase, are critical for the control of PIN polarity (Benjamins et al. 2001, Christensen et al. 2000, Friml et al. 2004, Michniewicz et al. 2007). Two important questions are how these kinases and phosphatases are regulated and how they are linked to the initial signal that specifies the cell polarity for PIN localization. Both PID and PP2AA partially colocalize with PIN1 in the PM, suggesting that the signaling most likely occurs there. A likely component in this signaling pathway(s) is 3-phosphoinositide-dependent protein kinase 1 (PDK1), which interacts with, phosphorylates, and activates PID (Zegzouti et al. 2006). An analogous signaling pathway involving PDK1 activation of PKC, a ser/thr kinase related to PID, modulates polarized PM localization of the GLUT4 glucose transporter in mammalian cells (Watson et al. 2004). There are also hints that ROP signaling may regulate PIN polarization (Jaillais et al. 2007; Li et al. 2005; Xu & Scheres 2005a,b). GFP-ROP2 localization is polarized in a similar manner to PIN2-GFP, and ROP2 overexpression increases PIN2-GFP polar localization (Li et al. 2005). VPS29-mediated endosome sorting is required for PIN1 repolarization to initiate lateral root formation, and the lateral root formation defect in *vps29* is epistatic to CA-ROP2 induction of lateral root formation (Jaillais et al. 2007). Given these results, it will certainly be worthwhile to test the role of ROP signaling in PIN polarization.

Asymmetric Cell Division: The Guard Cell Model

Signaling to cell polarity formation required for asymmetric cell division in plants may be the least understood polarity system. However, a potential important breakthrough in this area is a series of recent discoveries of the signaling mechanisms regulating guard cell differentiation. The first discovery is the identification of the *TOO MANY MOUTHS* (*TMM*) gene, which encodes an extracellular leucine-rich repeat (LRR) receptor–like protein (Nadeau & Sack 2002). Loss of *TMM* function mutations causes a number of stomatal patterning pheno-types including stomata clustering, suggestive of a defect in responding to positional signals. It was later shown that a triple-knockout mutant for three closely related RLKs, ERRECTA and two ERRECTA-like genes (*ERL1* and *ERL2*), exhibits a *tmm* phenotype and is epistatic to *tmm* (Shpak et al. 2005). A recent report has identified a secreted peptide (EPF1) that genetically acts in the same pathway as TMM and the ERRECTA family (Hara et al. 2007). Interestingly, *EPF1* is only expressed in guard cell precursor cells, whereas both *TMM* and the *ERRECTA* family are expressed in their neighboring cells (Hara et al. 2007, Nadeau & Sack 2002, Shpak et al. 2005). Thus, it was proposed that the secreted EPF1 diffuses to the neighbors of the precursor cells to activate the coreceptor of TMM and the ERRECTA family proteins to regulate asymmetric cell division. Other exciting reports show that a mitogen-activated protein kinase (MAPK) cascade, composed of YODA (a MAPKKK), MKK4/MKK5, and MPK3/MPK6, has a function similar to that of TMM, ERRECTA, and EPF1 and appears to act downstream of them (Bergmann et al. 2004, Lukowitz et al. 2004, Wang et al. 2007). The constitutive activation of the MAP cascade causes an apparent defect in asymmetric cell division, turning all guard cells into PC (Lukowitz et al. 2004, Wang et al. 2007). The absence of this cascade apparently also abolishes the asymmetric cell division of the zygote, resulting in the elimination of suspensor cell fate (Lukowitz et al. 2004, Wang et al. 2007). Therefore, this apparent positional signal–mediated pathway seems to control the polarity of cell division in *Arabidopsis*.

However, there is an alternative hypothesis that this pathway acts downstream of the polarity signaling to regulate cell fate rather than cell polarity per se. In support of the polarity hypothesis, the loss-of-function *yoda* mutant has a defect in zygote cell elongation, which is

likely a defect in zygote polarity establishment (Lukowitz et al. 2004, Wang et al. 2007). Unfortunately, the lack of cell polarity markers for guard cell stem cells or zygotes makes it difficult to directly test the role of this pathway in cell polarity control. Assuming that this signaling pathway regulates the polarity of cell division, some interesting questions remain to be answered. What is the cellular signaling target: polarization of the cytoskeleton, positioning of the nucleus, or polar cell expansion? Is this or another polarity-signaling pathway linked to the control of asymmetric cell division by intrinsic transcriptional factors known to affect asymmetric cell division (Aida et al. 2004, Pillitteri & Torii 2007, Xu et al. 2006)? Do similar pathways regulate asymmetric cell division in other aspects of plant development? What is the connection between RLKs and the MAPK cascade? Given the widespread role of ROPs in cell polarity control and the interaction of RopGEFs with RLKs (see above), it is tempting to speculate that ROP may be involved in bridging this gap.

CONCLUSIONS AND FUTURE PROSPECTS

Investigators have made important breakthroughs in studies of cellular signaling and cell polarity formation, using several plant model systems. In polarized cell growth, localized and dynamic ROP GTPase activity controls polarity establishment through the regulation of the cytoskeleton and vesicular trafficking, providing a framework for addressing many important and interesting questions in cell polarity control. It is yet to be determined whether this signaling paradigm can be extended to other cell polarity systems such as zygote polarization, polar PIN localization, and asymmetric cell division. Cytoskeleton-mediated feedback loops have been implicated in the regulation of localized and dynamic ROP signaling, but it is unclear what the molecular details of the feedback loops are and how they are triggered by an initial polarizing cue. Protein kinases such as plant-specific CDPKs and the conserved PID-related protein kinase family are known to participate in polarity signaling, but it is unknown whether and how they are linked to ROP signaling. PIPs have been implicated in cell polarity regulation in plants, yet their precise role and connection to ROP signaling need to be investigated. Although vesicular trafficking mechanisms, which include processes such as exocytosis and endocytic recycling, are critical for polarity formation, how they are linked to polarity signaling mechanisms remains vague. With existing and expanding genetic tools and molecular and cytological markers combined with proteomic, biochemical, and molecular approaches, exciting insights into these outstanding questions are in sight.

SUMMARY POINTS

1. Cell polarity signaling has been studied through several model systems, including (*a*) single-cell systems such as PT and root hairs and (*b*) multicellular systems such as PC and PIN protein localization in roots.
2. As in animal cells, cortical MT are primarily involved in polarity induction, whereas the polarity-signaling targets, F-actin and associated vesicular trafficking, participate in polarity establishment and feedback-regulate polarity signaling.
3. As in all other eukaryotic cells, the plant subfamily of conserved Rho GTPases, ROP, is the central regulator of cell polarity signaling.
4. ROP is mediated by both conserved and plant-specific regulators, and it controls the dynamics and organization of both F-actin and MT through plant-specific effector proteins such as RICs.
5. In the PT single-cell system, the dynamics of the active ROP1 apical cap is essential for polarized cell growth: It modulates polarized exocytosis and is controlled by interlinking positive feedback–mediated lateral propagation and negative feedback–mediated RhoGAP-dependent global inhibition.

6. In jigsaw-puzzle-shaped PC, the complex polarity of cell expansion is controlled by ROP2 activation of localized F-actin in the lobing domain, which counteracts RIC1 activation of MT arrangement in the indenting domain.
7. Auxin may be a common polarizing signal, activating ROP signaling pathways in its control of cell polarity.
8. Polar localization of PINs is mediated by a cascade of PDK1 and PID ser/thr kinases, whereas asymmetric cell division may be controlled by an RLK-MAPK cascade signaling mechanism. Both of these kinase signaling cascades may be linked to ROP GTPase.

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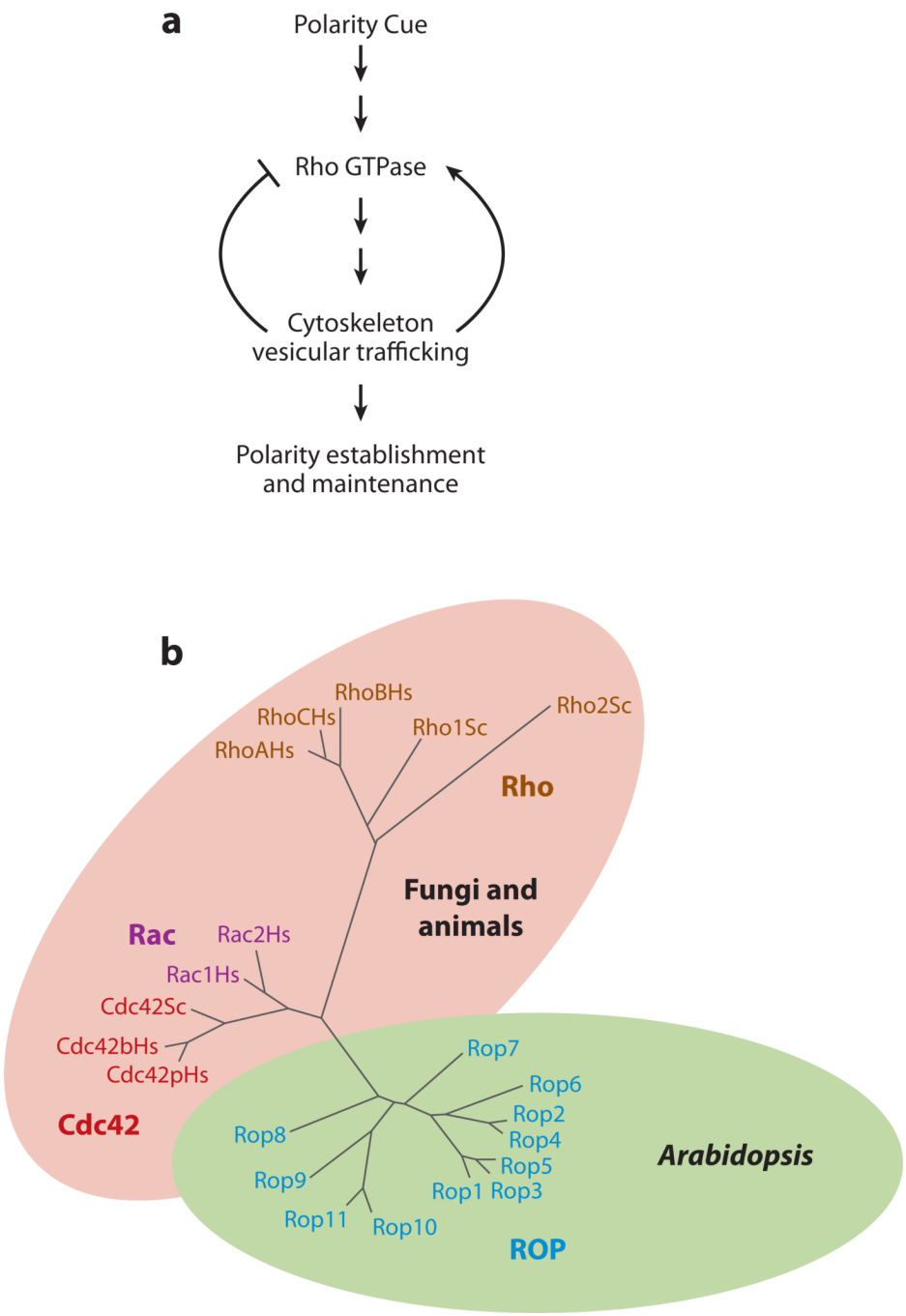

Figure 1.
A unifying principle underlying the formation of cell polarity in eukaryotic cells. (*a*) A general signaling mechanism for cell polarity formation. (*b*) A simplified phylogenetic tree showing the major subfamilies of the Rho family small GTPases. All 11 members of the *Arabidopsis* Rho-related GTPase from plants (ROP) family are shown, but only representative members of the Cdc42, Rac, and Rho subfamilies are included.

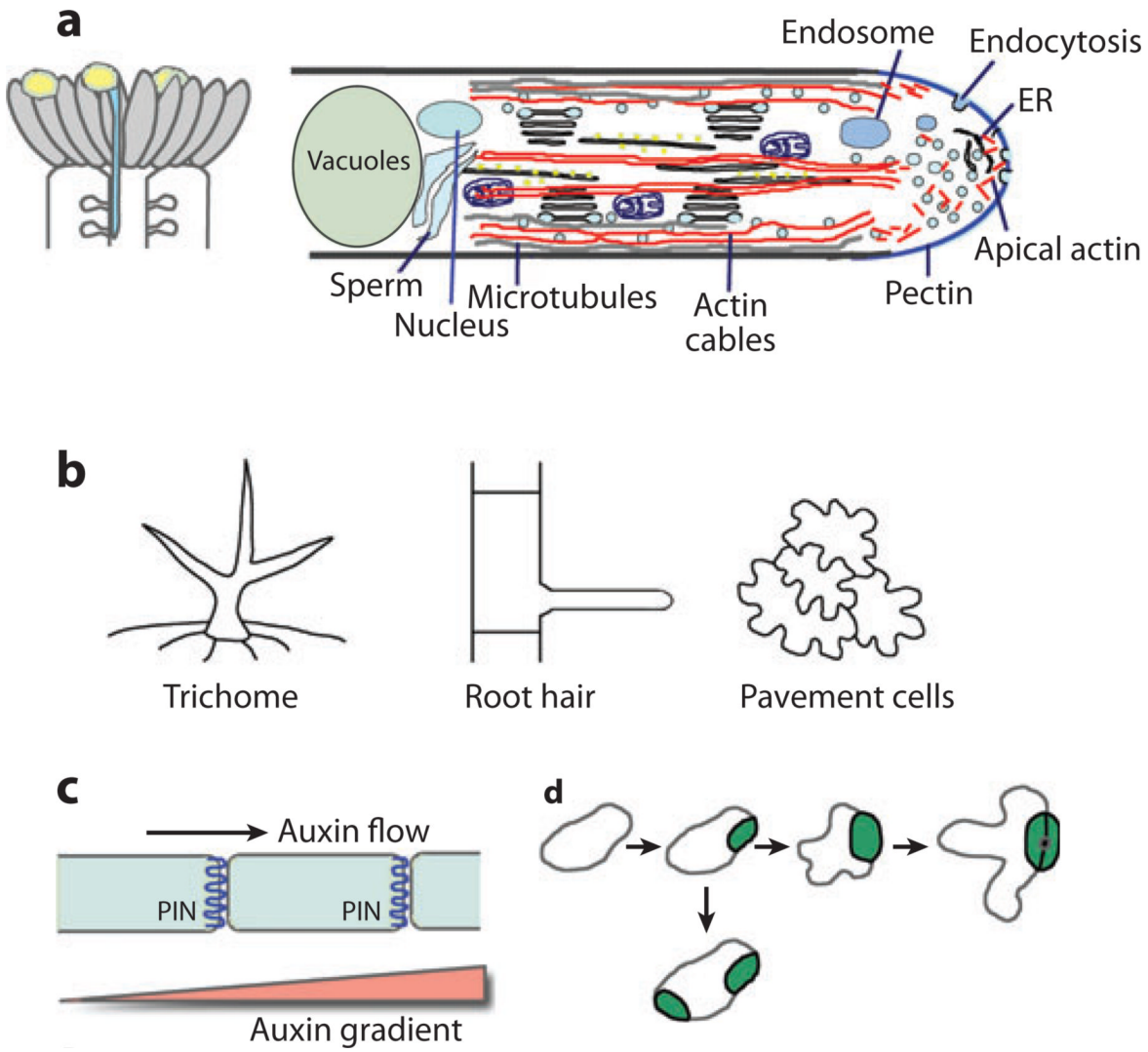

Figure 2.

Model cell polarity systems in plants. (*a*) Tip growth model: the pollen tube. (*Left*) A schematic of the top portion of a pistil with growing pollen tubes. (*Right*) A schematic showing the polarized cellular structure of pollen tubes. (*b*) Epidermal cells as a model for the study of planar cell polarity and/or the polarity of diffuse growth. (*Left*) Single-cell leaf trichome. (*Middle*) Trichoblast and root hairs. (*Right*) Pavement cells. (*c*) The polar localization of PINs (PIN-FORMED proteins), which directs auxin flow and produces auxin gradients, is used as a model for the study of asymmetric distribution of molecules within a cell. (*d*) Guard cell differentiation as a model for the investigation of the polarity of cell division.

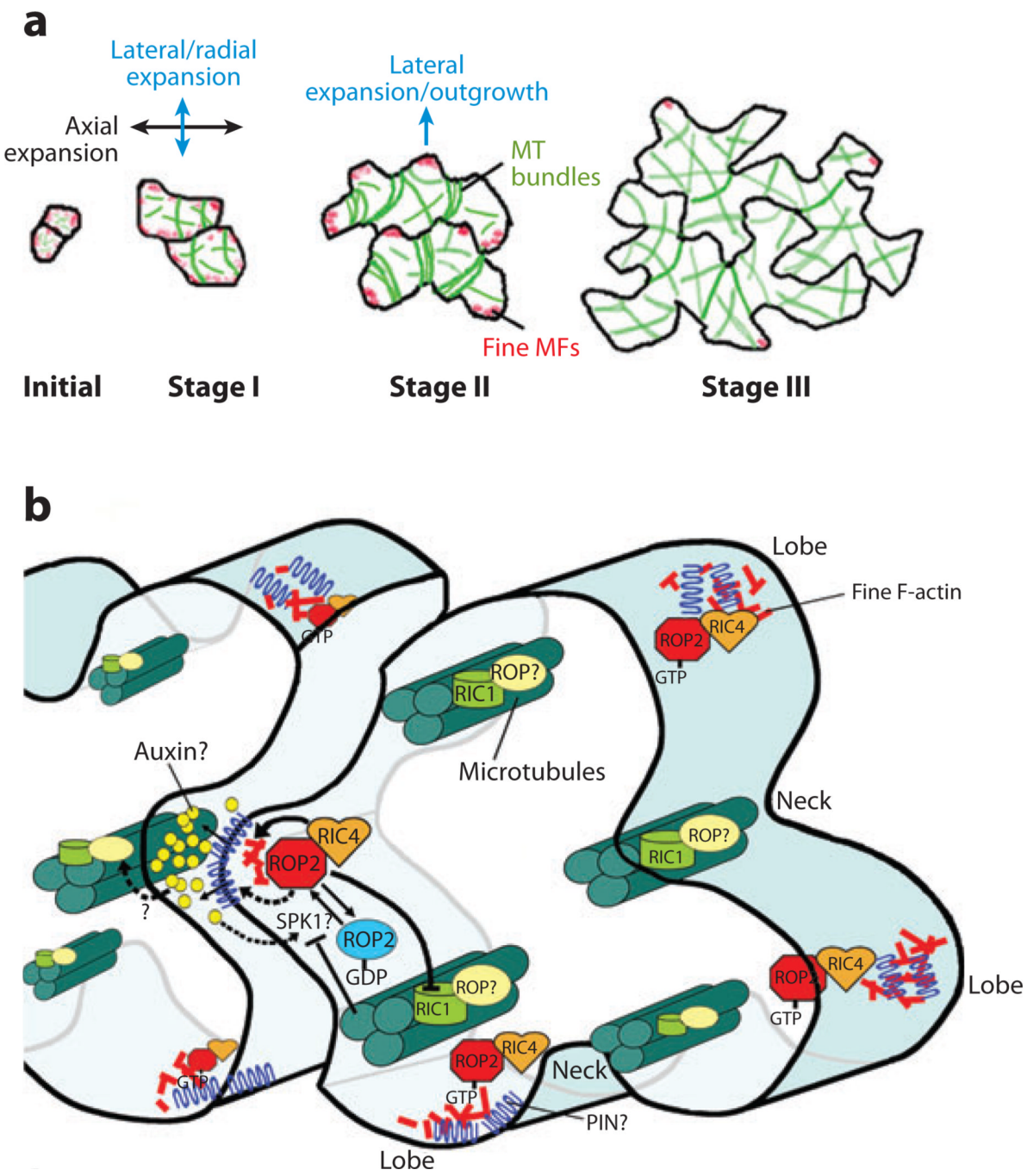

Figure 3.

A model for a signaling network regulating the formation of the jigsaw-puzzle appearance of *Arabidopsis* leaf pavement cells (PC). (*a*) The development of PC can be separated into three stages and is associated both with cortical fine actin microfilaments (F-actin) (*red patches*) and with microtubules (MT) (*green lines*) (Fu et al. 2002, 2005). Near-square PC initials first elongate slightly to form near-rectangular cells (stage I), which produce alternating small bumps and indentations, generating cells with multiple shallow lobes and indentations (also termed sinuses or necks) (stage II). Reiterative outgrowing and indenting continue, producing highly lobed interlocking PC that often contain secondary lobes (stage III). From Fu et al. (2005) with permission. (*b*) A model for the ROP GTPase–dependent signaling mechanism

for PC morphogenesis. This model includes known components (ROP2, RIC4, F-actin, RIC1, MT, SPK1), speculative factors (auxin and a PIN protein), and their interactions in the ROP (Rho-related GTPase from plants)-signaling network underlying PC morphogenesis. Solid arrows indicate pathways well supported by experiments. Dotted arrows indicate speculative steps/pathways. ER denotes endoplasmic reticulum.

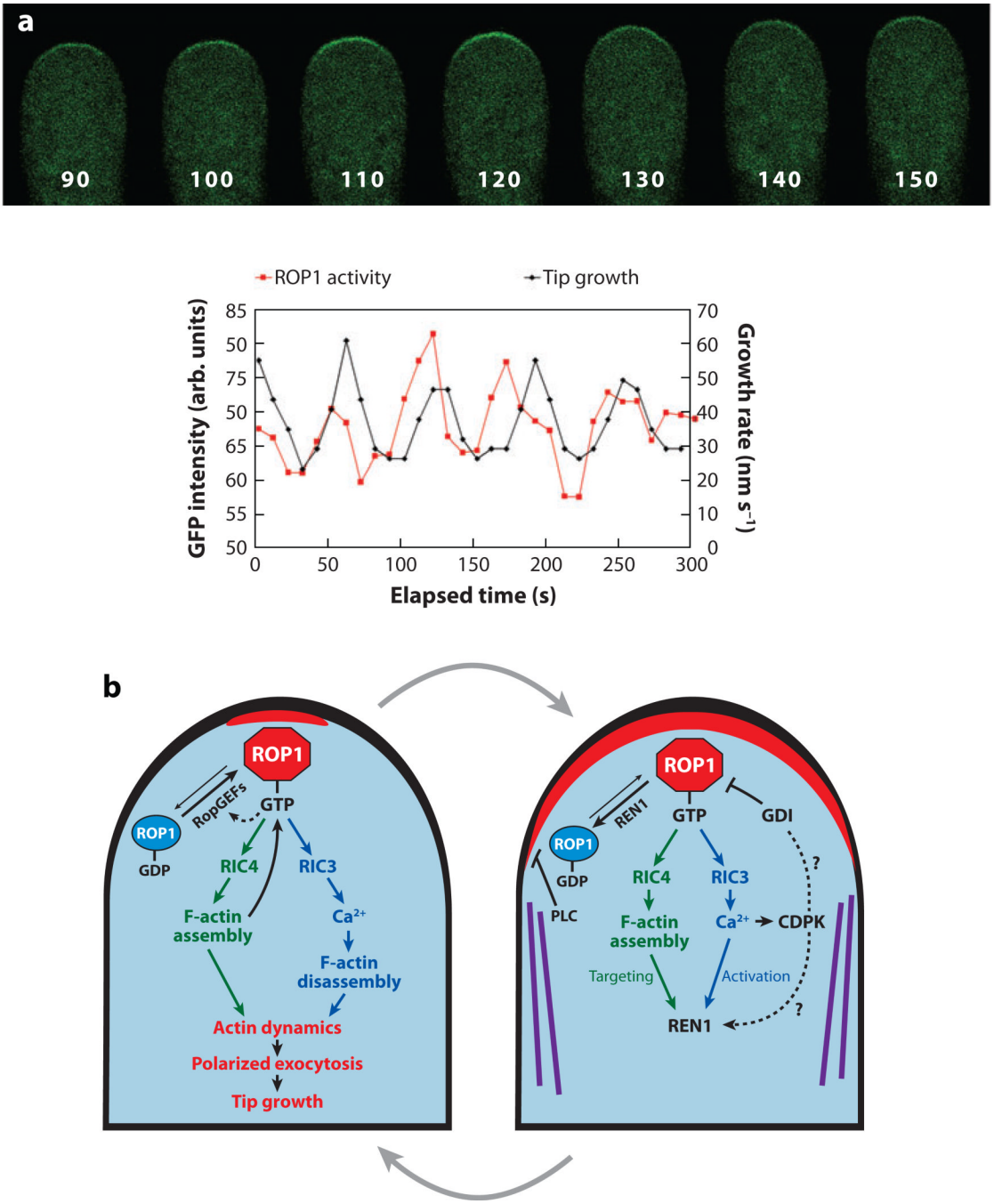

Figure 4.

A model for the generation and maintenance of the dynamic and oscillating apical cap of active ROP1 (where ROP refers to Rho-related GTPase from plants) in pollen tubes. (*a*) A time series of images showing the localization of a green fluorescent protein (GFP)-based active ROP1 reporter in a tobacco pollen tube transiently expressing this marker, i.e., GFP fused to the N terminus of a RIC4 deletion mutant (Hwang et al. 2005). Numbers at the bottom of each pollen tube image indicate seconds in the time series shown in the graph at the bottom of this figure. (*Bottom*) Graphs show the oscillation of plasma membrane (PM)-localized GFP-RIC4ΔC. Modified from figure 3 of Hwang et al. (2005). (*b*) Models for positive feedback–mediated lateral propagation of the apical ROP1 activity and for negative feedback–mediated global

ROP1 downregulation by the REN1 GTPase–activating protein (RhoGAP) and lateral inhibition by cortical microtubules (MT) and phospholipase C (PLC). Solid arrows indicate pathways supported by experimental evidence. Dotted arrows indicate speculative pathways lacking experimental support. Abbreviations used: CDPK, calcium-dependent protein kinase; F-actin, actin microfilaments; RIC, ROP-interactive Cdc42/Rac-interactive binding (CRIB) motif–containing protein.

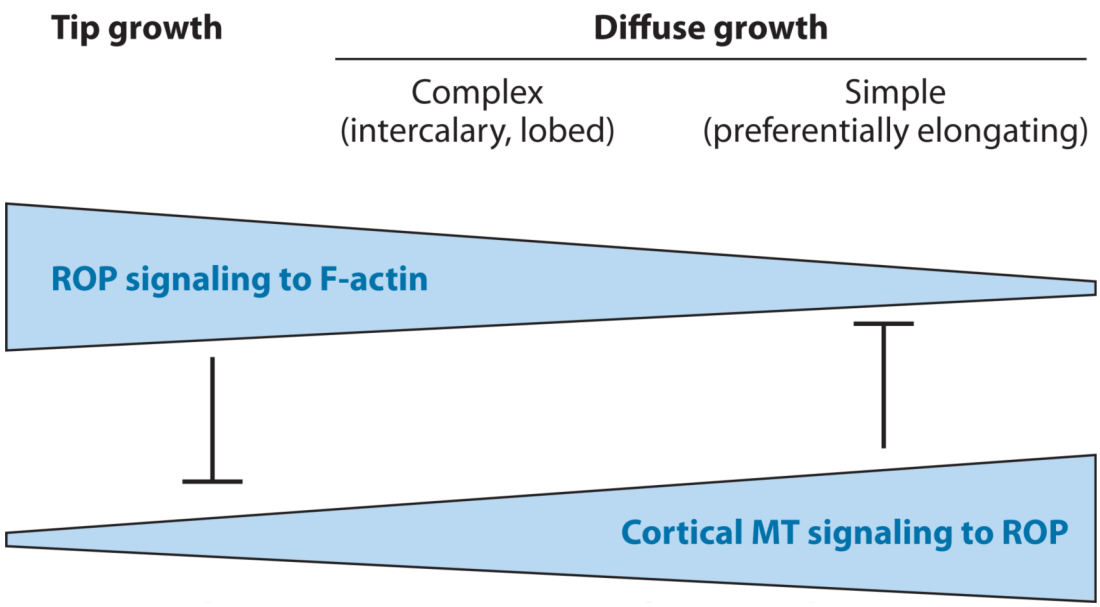

Figure 5.
A schematic model for a unifying concept of the mechanisms underlying the control of cell morphogenesis through tip growth or polarized diffuse growth. I propose that the primary mechanism for the control of cell polarity in tip growth is the tip-localized ROP (Rho-related GTPase from plants) activation of actin dynamics, which promotes targeted exocytosis, whereas microtubule (MT)-mediated lateral inhibition plays a secondary role in polarity control. In contrast, MT-dependent mechanisms play a primary role in the control of cell polarity of diffuse growth, whereas ROP activation of actin microfilaments (F-actin) plays a secondary role in polarity control. Cortical MT–mediated polarity control involves its role in aligning cellulose microfibrils and suppression of the ROP-actin pathway. The ROP-actin pathway and the cortical MT counteract one another to fine-tune cell polarity and cell shape formation.