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Invited Expert Review

Cell polarity: Regulators and mechanisms in plants[FA]

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Abstract Cell polarity plays an important role in a wide range of biological processes in plant growth and development. Cell polarity is manifested as the asymmetric distribution of molecules, for example, proteins and lipids, at the plasma membrane and/or inside of a cell. Here, we summarize a few polarized proteins that have been characterized in plants and we review recent advances towards understanding the molecular mechanism for them to polarize at the plasma membrane. Multiple mechanisms, including membrane trafficking, cytoskeletal activities, and protein phosphorylation, and so forth define the polarized plasma membrane domains. Recent discoveries suggest that the polar positioning of the proteo-lipid membrane domain may instruct the formation of polarity complexes in plants. In this review, we highlight the factors and regulators for their functions in establishing the membrane asymmetries in plant development. Furthermore, we discuss a few outstanding questions to be addressed to better understand the mechanisms by which cell polarity is regulated in plants.

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INTRODUCTION

Cell polarity, referring to the asymmetric distribution of cellular components, structure and function within a cell, is a fundamental feature of all living organisms and plays critical roles in almost all aspects of cellular function, for example, expansion, division, differentiation, growth, and morphogenesis (Campanale et al. 2017; Chiou et al. 2017). The plant cells display a diverse array of polarity underlying growth and patterning in development (Yang 2008; Qi and Greb 2017). For example, the pollen tube and root hair are formed by extremely polarized tip growth (Guan et al. 2013; Mendrinna and Persson 2015). The puzzle-shaped pavement cells require diffused polar growth for morphogenesis (Guimil and Dunand 2007; Qian et al. 2009). Specialized cell function, including directional movement of nutrient or phytohormones, can be achieved by directional enrichment and/or activity of the transporters (Yoshinari and Takano 2017). Cell polarity also plays important roles in the regulation of asymmetric cell division (ACD) (Shao and Dong 2016; Zhang and

Dong 2018; Muroyama and Bergmann 2019), an important biological process that generates two daughter cells that differ in cell fates and is essential for the development of multicellularity while maintaining the stem cell population in plants.

The plant cells possess numerous unique features, including the cell walls, that function to assist in the establishment of cellular asymmetry (De Smet and Beeckman 2011). One of the major mechanisms is to place key regulators, for example, proteins or lipids, to one side of the cell and this process often requires highly coordinated activities of cell signaling, membrane trafficking, and cytoskeleton reorganization. With regard to polarly localized proteins, they can be integral to the plasma membrane (PM) or associated with the PM. For the integral membrane proteins, to reach the PM, they are first synthesized in the endoplasmic reticulum (ER), followed by vesicle delivery along the secretory pathway through the Golgi apparatus and the trans-Golgi network (TGN), and finally reach the PM by exocytosis and vesicle fusion (Wang et al. 2017b). Many proteins are dynamically regulated at the plasma membrane where they play their biological function, while are also endocytosed via the clathrine-dependent and/or -independent pathways (Chen et al. 2011; Zhang et al. 2019). The destinations of the endocytosed PM proteins include being recycled back to the PM and/or delivered to the lytic vacuole for degradation (Jurgens 2004). The polarization of the PM proteins involves combined activities of targeted protein secretion, endocytosis, and/or endosomal recycling with the direction guided by external cues (Luschnig and Vert 2014; Langowski et al. 2016). On the other hand, the polarization of membrane-associated proteins requires the establishment of local membrane domain with distinct signatures that can be defined by specific biochemical or unique mechanical features (Hepler et al. 2013; Mangano et al. 2016). Also, the endosomes and their coordinated activities seem to be tightly integrated into the polarization machinery to polarize both membrane-embedded and -associated proteins.

In this review, we summarize the identified polarity factors and the key regulators in the establishment and maintenance of polarized membrane domains in plant cells. We give significant consideration of the endomembrane system and try to understand how dynamic membrane trafficking drives protein polarization in plants.

MAJOR POLARITY PROTEINS AND THE CELL SYSTEMS

The asymmetric distribution of proteins at the PM is an important feature of cell polarity in plants (Dettmer and Friml 2011). A few well-recognized such proteins include the auxin efflux carrier PIN-FORMED (PIN) proteins and some of their regulators (Wisniewska et al. 2006), the boron transporters NIP5;1 and BOR1 for nutrient uptake in the roots (Yoshinari and Takano 2017), the small GTPase ROPs in polarized cell growth (Yang 2008), and the scaffold proteins BASL and POLAR in stomatal development (Guo and Dong 2019). By specialized subcellular localization, they play important roles to regulate specific biological processes in plant development and growth.

Polarized PINs drive directional auxin flow

Auxin is unique among all phytohormones because it regulates numerous aspects of plant growth and development via its polar transport (Leyser 2018; Gallei et al. 2019). Based on molecular genetic studies in the model plant *Arabidopsis*, the tightly controlled auxin flow and distribution control the embryonic axes (Friml et al. 2003), the formation of primary and lateral roots (Sabatini et al. 1999; Benkova et al. 2003), shoot-derived organ generation (Benkova et al. 2003), and fruit development (Sorefan et al. 2009). At the cellular level, auxin signaling is also important for cell division patterning and morphogenesis (Xu et al. 2014; Smit and Weijers 2015). Auxin gradients are established and maintained mainly by the PIN efflux carriers that are polarly localized to drive directional auxin flow (Wisniewska et al. 2006; Adamowski and Friml 2015). The *Arabidopsis* genome encodes eight PIN proteins and five of them, including PIN1, PIN2, PIN3, PIN4, and PIN7, showed polarization at the PM in a cell type-specific manner and associated with specific developmental stages (Vieten et al. 2007; Adamowski and Friml 2015). Polarization of PIN proteins have been well characterized in a few cell systems in plant development. During early embryogenesis in *Arabidopsis*, the polarization of PIN1, PIN4, and PIN7 directs the auxin accumulation towards distinct parts of the developing embryo that results in the specification of the apical–basal plant axis and the division patterning (Figure 1A). In the root, the PIN proteins are differentially polarized at different cell layers and the coordinated directions of PIN polarization drives directional auxin flow

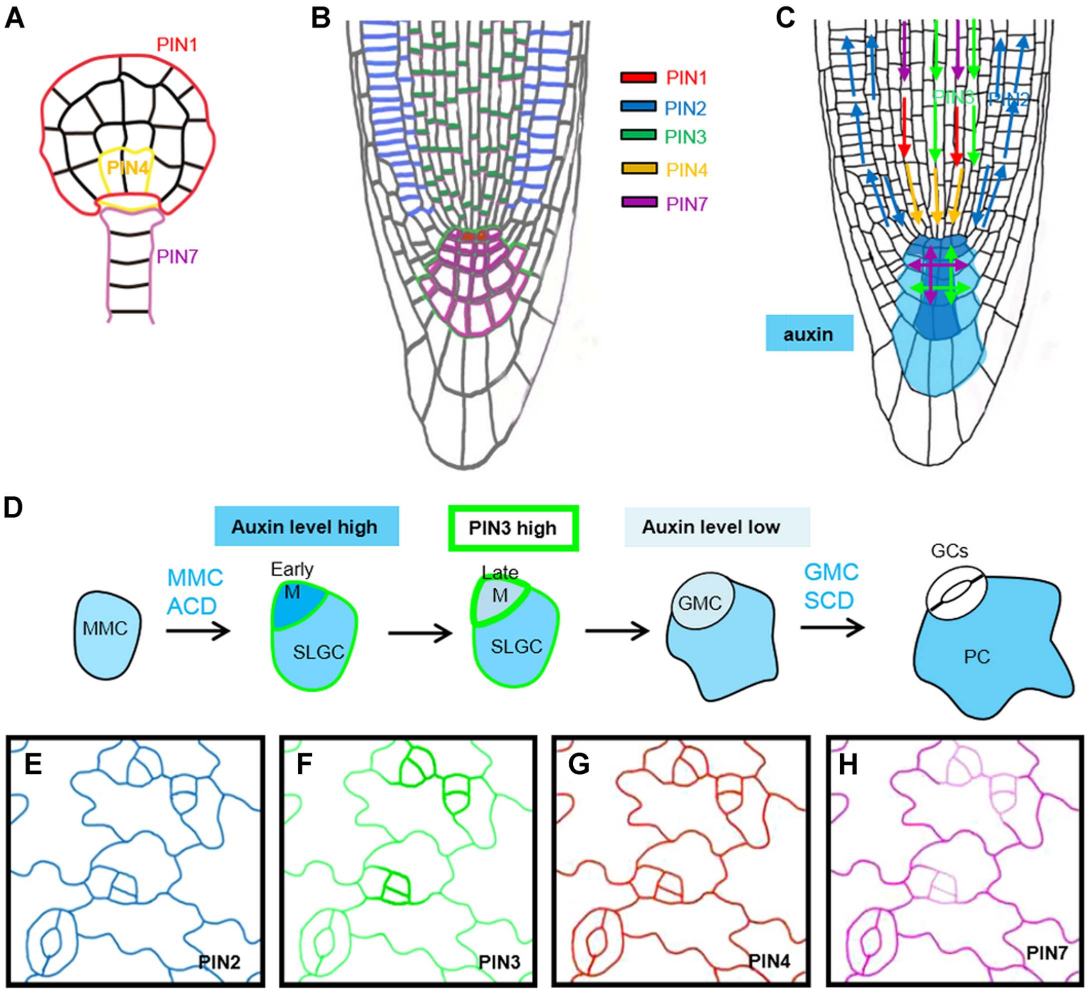

Figure 1. PIN distribution and polarization in the plant development
(**A**) PINs expression and polarization pattern in the globular stage of *Arabidopsis* embryo. (**B**) PINs expression and polarization pattern in the *Arabidopsis* root tip. (**C**) Distribution of PIN proteins contributes to the auxin gradients in the root tip. (**D**) Differential PIN3 and auxin activity levels during the process of stomatal development. (**E–H**) Differential expression patterns of PIN2, PIN3, PIN4 and PIN7 in the epidermis of *Arabidopsis* cotyledon. Note, high levels of PIN3 and low levels of PIN7 in the M cells. (**E–H**), confocal images processed by photoshop to demonstrate differential expression levels of PIN proteins.

(Kleine-Vehn et al. 2008) (Figure 1B, C). PIN3 and PIN7 are expressed in the columella cells, where they regulate root gravitropism (Kleine-Vehn et al. 2010).

Polarized proteins and asymmetric cell signalling in stomatal development

Stomata are epidermal pores that allow gas exchange between plants and the atmosphere. Although monocots and dicots have distinctly structured stomatal complexes, 4-celled versus 2-celled, respectively, the formation of both types requires highly regulated asymmetric cell division (ACD) that generates daughter cells with distinct cell fates. In *Arabidopsis*, the initiation of stomatal cell lineage begins with the asymmetric division of meristemoid mother cells (MMCs), each of which divides to create a smaller meristemoid (M) and a larger stomatal lineage ground cell (SLGC) (Dong et al. 2009). After an ACD, the two daughter cells acquire different cell fate: the meristemoid ultimately turns into a pair of guard cells, while the SLGC retrieves from stomatal differentiation, but undergoes spacing divisions before becoming a pavement cell (Lau and Bergmann 2012; Shao and Dong 2016).

By using *Arabidopsis* as a genetic model system, both auxin signaling and protein expression showed asymmetries that regulate stomatal division and cell fate determination. First of all, auxin levels were found to dynamically fluctuate at different stages of stomatal development (Le et al. 2014). Auxin signaling peaks in the young meristemoids, whereas drops when a meristemoid is differentiating into a guard mother cell (GMC) (Le et al. 2014). The depletion of auxin levels in the Ms was believed to trigger the transition of cell division from stem cell-like ACD to the GMC symmetric division followed by terminal guard cell differentiation. Interestingly, among the four PIN proteins expressed in the leaf epidermal cells (PIN2, 3, 4, and 7), the dynamic expression levels of PIN3 are most closely correlated with the dynamic auxin signaling (Le et al. 2014). Although the other PIN protein show differential expression patterns (Figure 1D–G), their combined function is critical for stomatal development and patterning because in *pin* higher-order mutants, *pin2;3;4;7,* stomata form clusters (Le et al. 2014).

During the past decade or so, significant progress has been made regarding the identification of polarized proteins and their function in the regulation of stomatal ACD in *Arabidopsis*. The plant-specific protein, BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL), was first identified for its function in stomatal ACD by cortical polarization (Dong et al. 2009). The loss-of-function *basl* mutants produce stomatal divisions being pronouncedly symmetric, leading to both daughter cells become stomata that are in direct contact. Before an ACD, the GFP-tagged BASL protein accumulates in the nucleus and polarizes at the cortical PM to direct division orientation. After an ACD, the BASL polarity is only inherited to the SLGC where it assists in the daughter cell fate specification (Dong et al. 2009) (Figure 2A). The functions of polarized BASL was connected to MAPK signaling by locally concentrating the components of the signaling cassette, the MAPKKK YODA (YDA) and MAPK 3 and 6, for suppressing SPCH thus stomatal fate differentiation (Zhang et al. 2015, 2016). The BREVIS RADIX (BRX) proteins were identified as physical partners of BASL and their cortical polarization recapitulated that of BASL in the stomatal lineage cells (Bringmann and Bergmann 2017). Previously, the BRX genes were found to regulate root development by basally polarizing in the root phloem vasculature (Scacchi et al. 2009), where BRX may suppress the D6 protein kinase-related kinase PAX and its activation of PIN-mediated auxin efflux (Marhava et al. 2018). However, how the BRX family functions in stomatal development remains unknow. In addition, POLAR LOCALIZATION DURING ASYMMETRIC DIVISION AND REDISTRIBUTION (POLAR) is another plant-specific protein in the stomata lineage and its polarization depends on the presence of BASL (Pillitteri et al. 2011). With no evidence supporting that BASL and POLAR directly interact, POLAR appears to regulate ACDs by recruiting BIN2 and other GSK3-like kinases to the polarity crescent, where BIN2 suppresses the YDA MAPK module, so that stomatal ACD can be enabled (Houbaert et al. 2018). Thus, the BASL-centered polarity complex, by recruiting POLAR and BIN2, may help to promote the division potential before an ACD and, by elevating the YDA MAPK signaling, may specify the SLGC to become a

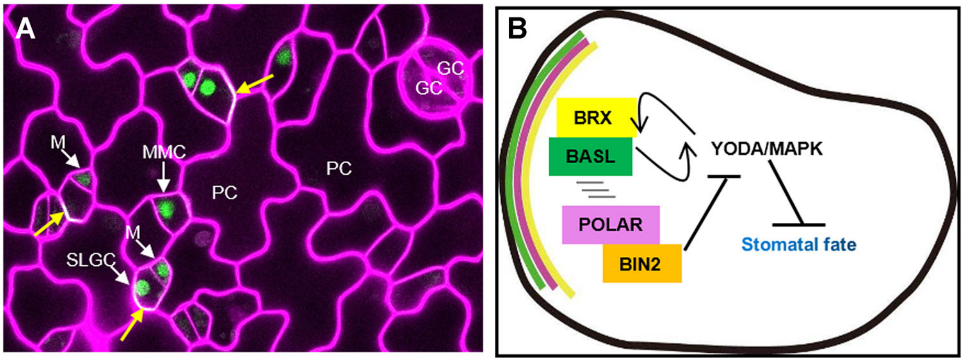

Figure 2. Cell polarity drives asymmetric cell division during stomatal formation in *Arabidopsis* cotyledon
(**A**) Confocal image to show GFP-BASL expression in the *Arabidopsis* cotyledon. MMC: meristemoid mother cell; M: meristemoid; CG: Guard cell; SLGC: Stomatal lineage ground cell; Green indicates GFP-BASL and magenta marks cell outlines. Yellow arrowheads indicate the sites of GFP-BASL polarization. (**B**) Molecular components and genetic interactions of the BASL/POLAR polarity module in the regulation of stomatal asymmetric cell division.

non-stomatal pavement cell after an ACD (Figure 2B). Interestingly, all of the polarity proteins identified in the stomatal lineage cells so far are associated with the PM, therefore their polarization was hypothesized to rely on protein–protein and/or protein–lipid interactions.

In monocots, a stomatal complex consists of four cells including two GCs and two subsidiary cells. The formation of such a stomatal complex involves a series of coordinated ACD. A stomatal precursor cell undergoes an ACD to generate a GMC, which may release unknown signals to induce the neighboring cells in contact to become the subsidiary mother cells (SMCs) (Facette and Smith 2012). The SMCs subsequently divide asymmetrically to generate subsidiary cells flanking the GMC, and the GMC then divides longitudinally to form a pair of guard cells. Much knowledge has been gained through the studies of the SMC asymmetric divisions in *Zea mays* (Figure 3) (Facette and Smith 2012; Pillitteri and Torii 2012). Genetic screens identified Pangloss1 (PAN1) and PAN2, the two genes encoding leucine-rich repeat receptor-like proteins (LRR-RLKs) that function cooperatively to polarize the SMC divisions (Cartwright et al. 2009; Sutimantanapi et al. 2014). Mutations of either gene resulted in similar phenotypes of defective SMC divisions and abnormal subsidiary cells. The PAN1 and PAN2 proteins co-polarize in the premitotic SMCs at the site of GMC contact to promote the formation of actin patches, which seemed to drive directional migration of the SMC nuclei (Sutimantanapi et al. 2014). PAN1 interacts with the type I Rho of Plants (ROP) GTPases to promote SMC polarization (Humphries et al. 2011). Downstream of the ROP signaling, the Brk1 gene encodes a small subunit of the SCAR/WAVE regulatory complex (WRC) that may activate the ARP2/3-mediated actin nucleation (Facette et al. 2015). But interestingly, the polarization of SCAR/WAVE appears prior to that of PANs and ROPs, suggesting asymmetric organization of the actin network might occur early (Facette et al. 2015). Thus, the crosstalk between PAN1/2 and the actin cytoskeleton establishes the SMC division to take place asymmetrically, but the detailed mechanism needs to be further investigated (Facette et al. 2015).

The establishment of cell fate asymmetry in the SMC ACD in monocots was found to be regulated by mobile transcription factors, for example, the bHLH MUTE in *Brachypodium* (Raissig et al. 2017). Interestingly, ZmMUTE is also mobile in maize, with the initial expression in the GMCs, and its function is required for both SMC polarization, differentiation and GMC divisions (Wang et al. 2019).

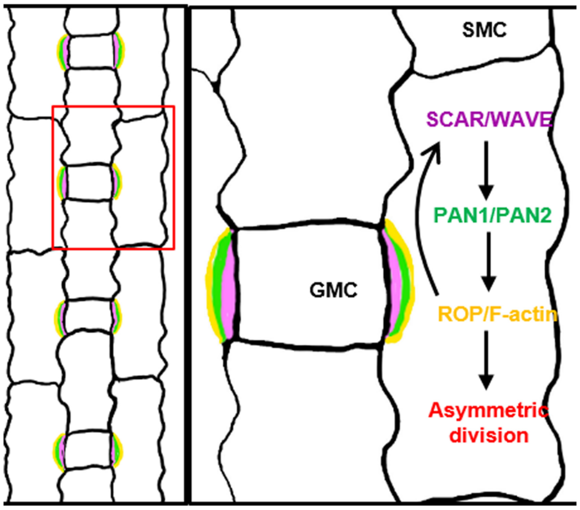

Figure 3. The PAN1/2 polarity module in the maize subsidiary mother cell (SMC) asymmetric cell division
In maize, before the SMC asymmetric division, the SCAR/WAVE regulatory complex first polarizes to the membrane adjacent to the guard mother cell (GMC) contact sites, where PAN2 polarizes subsequently. After PAN2 polarization, PAN1 and Rho of Plants (ROP) proteins are polarized, followed by the formation of an actin patch and the directional migration of the pre-mitotic SMC nucleus. Finally, the SMC undergoes asymmetric cell division. Purple lines indicate the site of SCAR/WAVE complex; green lines indicate where PAN1 and PAN2 proteins are polarized. The Orange lines indicate the locally enriched ROP6 molecules.

Rho of Plants GTPases are master regulators of cell polarity in plants

The Rho family of small GTPases are conserved master regulators in the establishment of cell polarity in all eukaryotic organisms. In plants, there is a single Rho-GTPase subfamily called Rho-like GTPases from plants (ROPs) (Yang and Fu 2007; Craddock et al. 2012) and their functions have been tightly associated with the cytoskeleton and vesicular trafficking (see reviews in Ying et al. 2003; Craddock et al. 2012). Plants have also evolved specific regulators, including ROP-Guanine Exchange Factors (GEFs) and the Rop-interactive CRIB motif-containing protein (RIC) effectors (Craddock et al. 2012). ROPs cycle between membrane-bound and cytosolic locations and are only active when associated with membranes via lipid modifications (Yalovsky 2015).

Arabidopsis genome encodes 11 ROPs. Members of the ROP subfamily have diverged to regulate distinct cellular functions (Vernoud, 2003 #133). ROP1–6 retain the conserved Rho-GTPase function of spatially controlling a variety of cellular processes by regulating the cytoskeleton and vesicular trafficking. ROP1, ROP3, and ROP5 may act redundantly in the modulation of tip growth in *Arabidopsis* pollen tubes (Li et al. 2001; Gu et al. 2006), whereas ROP2, ROP4, and ROP6 regulate polar cell growth and cell polarity in vegetative cells (Fu et al. 2005; Craddock et al. 2012).

NIP5;1 and BOR1 regulates boron homeostasis in plants

Boron is an essential element for plants but is toxic in excess. Therefore, plants must adapt to both limiting and excess boron conditions for normal growth (Yoshinari and Takano 2017). Under boron-limiting conditions, plants use boric acid channels of the major intrinsic protein (MIP) family and the BOR family of borate exporters for uptake and translocation of boron to support growth of various plant species (Takano et al. 2008). In *Arabidopsis*, NIP5;1 and BOR1 are located in the plasma membrane and polarized toward soil and stele, respectively, in various root cells for efficient transport of boron from the soil to the stele (Wang et al. 2017a; Yoshinari and Takano 2017) (Figure 4A).

Casparian strip polarized proteins

Casparian strip plays a critical role in sealing endodermal cells in the root to block uncontrolled extracellular uptake of nutrients and water (Doblas et al. 2017). In *Arabidopsis*, there are five Casparian strip membrane domain proteins 1 to 5 (CASP1–5) (Roppolo et al. 2011). The CASP1–GFP signal localizes at the plasma membrane precisely coincided with the Casparian strip itself. EXO70A1 plays a central role in the spatial organization of Casparian strip because *exo70a1* mutations specifically affected the localization, but not the secretion of CASP1-GFP (Kalmbach et al. 2017). The receptor-like kinase SGN3/GASSHO1 receives the signals from the Casparian strip integrity factor 1 (CIF1) and CIF2 to regulate the Casparian strip formation (Figure 4B) (Nakayama et al. 2017).

MOLECULAR MECHANISMS UNDERLYING PROTEIN POLARIZATION AT THE PM

The organization of the plasma membrane is highly complex and ever-changing. Polarized proteins residing at or in the PM of the cell, instead of statically staying, are often tightly regulated by dynamic membrane activities, including endocytosis, exocytosis, endocytic recycling that send the internalized proteins back to the PM, and/or vacuolar delivery for protein degradation (Figure 5). Endocytosis and endosomal trafficking are essential processes that control the dynamic turnover of plasma membrane proteins, which include transporters, cell surface receptors and cell wall regulators, and so forth. The PM proteins enter into the cell though endocytosis that is mediated mainly by clathrin-dependent and clathrin-independent mechanisms (Fan et al. 2015).

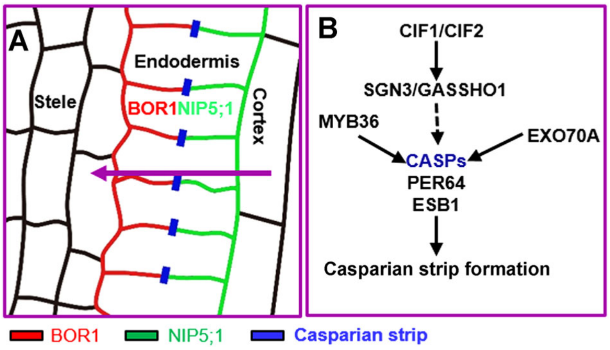

Figure 4. Polarized proteins driving directional nutrient uptake and Casparian strip formation, respectively, in the root

(**A**) Nutrient uptake (Boron) in the *Arabidopsis* root is driven by polarized transmembrane transporters, BOR1 and NIP5;1. Blue lines indicate Casparian strip. (**B**) The working model for key regulators in the generation of Casparian strip in the *Arabidopsis* root. Casparian strip membrane domain proteins (CASP)1 localizes at the plasma membrane precisely coincided with the Casparian strip. MYB36 directly and positively regulates the expression of the other Casparian strip genes *CASP1*, *PER64*, and *ESB1*. EXO70A1 affects the local enrichment of CASP1. The receptor-like kinase (SGN3/GASSHO1) receives the signals from the Casparian strip integrity factor 1 (CIF1) and CIF2 for the Casparian strip formation.

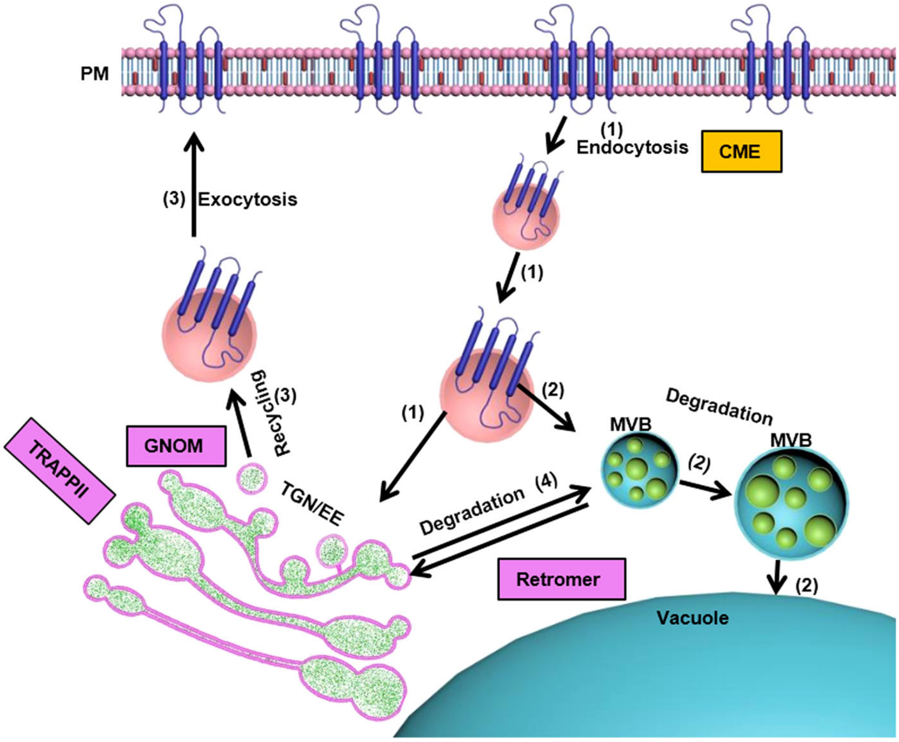

Figure 5. Membrane trafficking in the regulation of protein polarization
The plasma membrane (PM) proteins are internalized by the clathrine-mediated endocytosis (CME). Once endocytosed, they travel to the trans-Golgi-Network (TGN)/early endosomes (EE) (1) where they can be directed to the vacuole for degradation (2 and 4) or from TGN recycled back to the plasma membrane (3).

Endocytic recycling events rely on small GTPases-dependent and retromer-dependent pathways (Paez Valencia et al. 2016), by which cellular polarization, hormone and metal ion transport, and specialized developmental programs can be regulated.

The necessity of endocytosis for protein polarization

As in animal cells, the clathhrin-mediated endocytosis (CME) is the main entry point for extracellular materials and the PM proteins in plant cells (Paez Valencia et al. 2016). Also, the CME has a great influence on the polarization of PM proteins in plants that can be well exemplified by how the PIN proteins are polarized. The AP-2 (ADAPTOR PROTEIN2) complex is implicated in the plant CME process (McMahon and Boucrot 2011). In plants, when endocytosis is blocked by chemical inhibitors, the polarized PIN proteins were found to spread laterally (Kleine-Vehn et al. 2008). The laterally diffused PIN proteins outside of the polar domains can be recycled by the CME internalization followed by directional transcytosis for the recovery (Kleine-Vehn et al. 2008). Indeed, the polar PIN1-GFP localization during embryogenesis or the PIN2-GFP localization in the male reproductive organ development can be disrupted because of the impaired endocytosis caused by mutations in the AP-2 subunits, such as the σ adaptin (ap2σ) or the μ adaptin (ap2m) (Fan et al. 2013; Kim et al. 2013). In addition, both boron (B) transporters NIP5;1 and BOR1 require the AP2-controlled CME pathway to maintain their polarization at the PM (Yoshinari et al. 2016; Wang et al. 2017a). Interestingly, the BOR1 protein associates with the AP2 complex and under the low-B conditions, the AP2-dependent CME maintains the polar localization of BOR1 to support plant growth and face the challenge of boron deficiency (Yoshinari et al. 2019).

The SORTING NEXINS and VPS29 retromer complexes regulate cell polarity

Not all endocytosed membrane proteins are degraded in the vacuole. Some proteins escape vacuolar degradation from the endosomes by returning to the PM via the recycling pathways. The retromer complex with the core subunits Vps35, Vps29, and Vps26 and

SORTING NEXINS (SNXs) plays central roles in this process (Paez Valencia et al. 2016). In *Arabidopsis*, AtSNX1 and VPS29 were found to be essential for this recycling pathway. AtSNX1 defines a particular endosomal population, distinct from many other endosomal compartments, and PIN2 was found to accumulate to the AtSNX1 endosomal compartments under certain conditions (Jaillais et al. 2006). The functions of the retromer protein VPS29 was also linked to the regulation of cell polarity. The *Arabidopsis* mutant *vps29* displayed severe developmental defects in the formation of new axes in embryogenesis (Jaillais et al. 2007). Furthermore, VPS29 associates with the SNX1 endosomes and functions in the regulation of endosome homeostasis, PIN function in polar auxin transport and specifically required for PIN1 and PIN2 trafficking (Jaillais et al. 2007).

GNOM-mediated endocytic recycling

The *GNOM/EMB30* gene encodes a guanine nucleotide exchange factor (GEF) that activates the ADP-ribosylation factors (ARF) and is sensitive to Brefeldin A (BFA) (Niko Geldner et al. 2003). GNOM mediates auxin transport in both embryogenesis and post-embryonic organ development (Geldner et al. 2004). The loss-of-function *gnom* mutants bear aberrant cell shape and mis-orientation cell division planes (Shevell et al. 1994). The well-established role for the ARF GEF GNOM is to regulate the polar localization of PIN1 (Steinmann et al. 1999), because randomized PIN1 polarity in *gnom* mutant embryos was found (Geldner et al. 2004). Knocking out multiple PIN genes led to the phenotypes that very much resembled those of *gnom* mutants (Friml et al. 2003). GNOM was subcellularly localized to the Golgi apparatus and, upon the BFA disturbance, it translocated to the abnormally aggregated endosomal compartments (Naramoto et al. 2014). Considering that GNOM functions as an ARF GEF, some ARF GTPases are the possible candidates participating in this process. Indeed, in *Arabidopsis*, ARF1 was found to localize to the Golgi apparatus and endocytic organelles and, when *ARF1* is absent, the polar localization of PIN2 was affected (Xu and Scheres 2005). A mechanistic understanding about how GNOM and ARF function at the Golgi to control the PIN proteins to polarize at the plasma membrane is still lacking that should be further pursued in future studies.

The RAB GEF TRAPPII at the trans-Golgi network

The trans-Golgi network (TGN) in plant cells is seen as an independent organelle that rapidly associates and disassociates with the Golgi bodies. The TGN provides a protein sorting station where it functions as early endosomes (EE) to receive endocytosed materials from the PM that will be either transported to the vacuole or recycle back to the PM (Paez Valencia et al. 2016). Recently, the TGN has been identified as a key organelle for protein polar transportation to the PM in plants (Viotti et al. 2010). Around the TGN, the highly conserved TRAPPII complex, a tethering factor for vesicle trafficking, acts as a GEF for Rab11 (homolog of yeast Ypt31/32) in early Golgi trafficking (Thomas and Fromme 2016). In *Arabidopsis*, TRAPPII was localized to the TGN and functionally linked to RabA/Rab11 to regulate polar targeting of both the auxin efflux carrier PIN2 and the auxin influx carrier AUX1 in root tip cells (Qi et al. 2011).

Small GTPases are a group of hydrolase enzymes implicated in a broad range of cellular signaling events, including protein and cell polarization in plants (Kania et al. 2014). To establish cell polarity, the small GTPases, such as RAB and ARF, may regulate vesicular trafficking between intracellular compartments by recruiting coat protein complexes to the vesicle formation sites, organizing the cytoskeleton, and docking vesicles to the destination membranes (Nielsen et al. 2008). The conserved Rab GTPases organize intracellular membrane trafficking at three consecutive stages of vesicular transport: vesicle formation, vesicle motility, followed by the tethering of vesicles to target compartments preceding the membrane-fusion events (Molendijk et al. 2004). Different isoforms of Rab GTPases assemble specific Rab domains on organelle membranes to define the identity of the membrane compartments (Zerial and McBride 2001). The plant Rab family consists of eight subfamilies, designated AtRabA-H with six of them present in yeast (Rutherford and Moore 2002). The RabA branch contains almost half of the total Rab GTPases in *Arabidopsis* and the closest mammalian homologues are localized to apical recycling endosomes to assist in polarizing epithelial cells (Rutherford and Moore 2002). Consistently, the RabA2 subgroup was found to be associated with the recycling endosomes that regulate polarized PIN proteins at the PM (Rutherford and Moore 2002). Additionally, AtRabA4b was found to label a TGN compartment that is polarly distributed in the growing root hairs, where RabA4b promotes polarized secretion of cell wall components to support tip growth (Preuss et al. 2006).

Secretion and exocytosis for PM proteins to polarize

In exocytosis, the PM proteins and membrane-bound secretory vesicles are delivered to the cell membrane or the extracellular matrix through membrane fusion. The delivery of either newly synthesized or recycled materials back to the PM is connected to the maintenance of protein homeostasis at the PM. In this process, the exocyst, an octameric protein complex, mediates vesicle trafficking by tethering and spatially targeting of post Golgi vesicles to the PM. Each exocyst complex contains eight subunits that are conserved in yeast and mammals and functions in targeted vesicular trafficking for the establishment of cellular polarity (Polgar and Fogelgren 2018). All of the eight subunits were found in the *Arabidopsis* genome and some of them were found to contribute to protein polarization. The EXO84B subunit regulates the secretion of polarized NIP5;1 (Xu et al. 2014; Mao et al. 2016). Furthermore, the EXO70 family (23 members in *Arabidopsis*) is important for polar growth and plant development (Synek et al. 2006). EXO70A1 was shown to regulate the recycling of internalized PIN1 and PIN2 to polarize at the PM (Drdova et al. 2013). In addition, EXO70A1 generates transient positional information to polarize the key factor CASP1 and plays a central role in the formation of Casparian strip (Kalmbach et al. 2017).

The cytoskeleton networks

In plants, the cytoskeleton is the intracellular scaffolding on which polarity can be framed (Wasteneys 2000). Trafficking of vesicles and their polar deposition to the PM takes place along the cytoskeleton. Treatment of actin and microtubules with depolymerizing chemicals revealed that the cytoskeleton targets polarly localized proteins, such as PIN proteins (Kleine-Vehn et al. 2008). The MT associated protein CLASP interacts with SNX1 to retrieve PIN2 from the vacuolar degradation to the recycling pathway in *Arabidopsis* (Ambrose et al. 2013). However, recent results also showed that the polarized PIN2 distribution is likely independent of cytoskeleton-guided endomembrane trafficking (Glanc et al. 2019).

The polarization of ROPs was evident in a number of cell systems, for example, the pollen tube, root hair, and pavement cells (Craddock et al. 2012). The ROP effectors and feedback functioning in actin and microtubule dynamics have been extensively studied and reviewed (Gu et al. 2004; Yang 2008; Craddock et al. 2012; Oda and Fukuda 2013), therefore are not further discussed here. Recent progress also suggested that ROP GEF, the DOCK family of SPIKE1, has sophisticated feedback regulations with both actin and microtubule networks to enable polarized growth in the trichome (Yanagisawa et al. 2018).

Polar positioning of the proteo-lipid membrane

In yeast and animal cells, the sterol composition regulates the asymmetric localization of some PM proteins (Makushok et al. 2016). In plants, the sterol-rich, detergent-resistant membrane domains, also called "lipid rafts", were found to provide mechanisms for protein localization and polarized function (Fischer et al. 2004). Previous studies showed that the mutants displaying cell and tissue polarity have defects in sterol composition, glycosylphosphatidylinositol-anchored proteins, glycosylphosphatidylinositol biosynthesis and phospholipid signaling (Fischer et al. 2004). Correct sterol composition is required for both auxin transport polarity at the organ level and polar positioning of the efflux carriers at the subcellular level (Willemsen et al. 2003).

Lipid rafts have long been proposed to functionally segregate proteins and lipids within different local membrane compartments, thereby regulating their interactions (Simons and Toomre 2000; Lingwood and Simons 2010). Lipid rafts may provide dynamic scaffolding for a variety of cellular processes, including cell signaling, stress responses, and membrane trafficking. In 2000, the first plant "lipid rafts" were isolated from tobacco leaf cells (Peskan et al. 2000). Recent studies showed that sterols and sphingolipids in lipid rafts function to regulate nanoclustering of small GTPases, such as Ras, Cdc42 and ROP6 (Platre et al. 2019; Xue et al. 2019). It was shown that the levels of the phospholipid phosphatidylserine modulate the number of ROP6 nanoclusters, so that ROP6 can be immobilized in the membranes (Platre et al. 2019). The regulation of this process was also linked to auxin signaling in root epidermal cells (Platre et al. 2019). Consistently, in leaf epidermal cells, auxin triggers the TMK1 receptor protein to form nanoclusters with

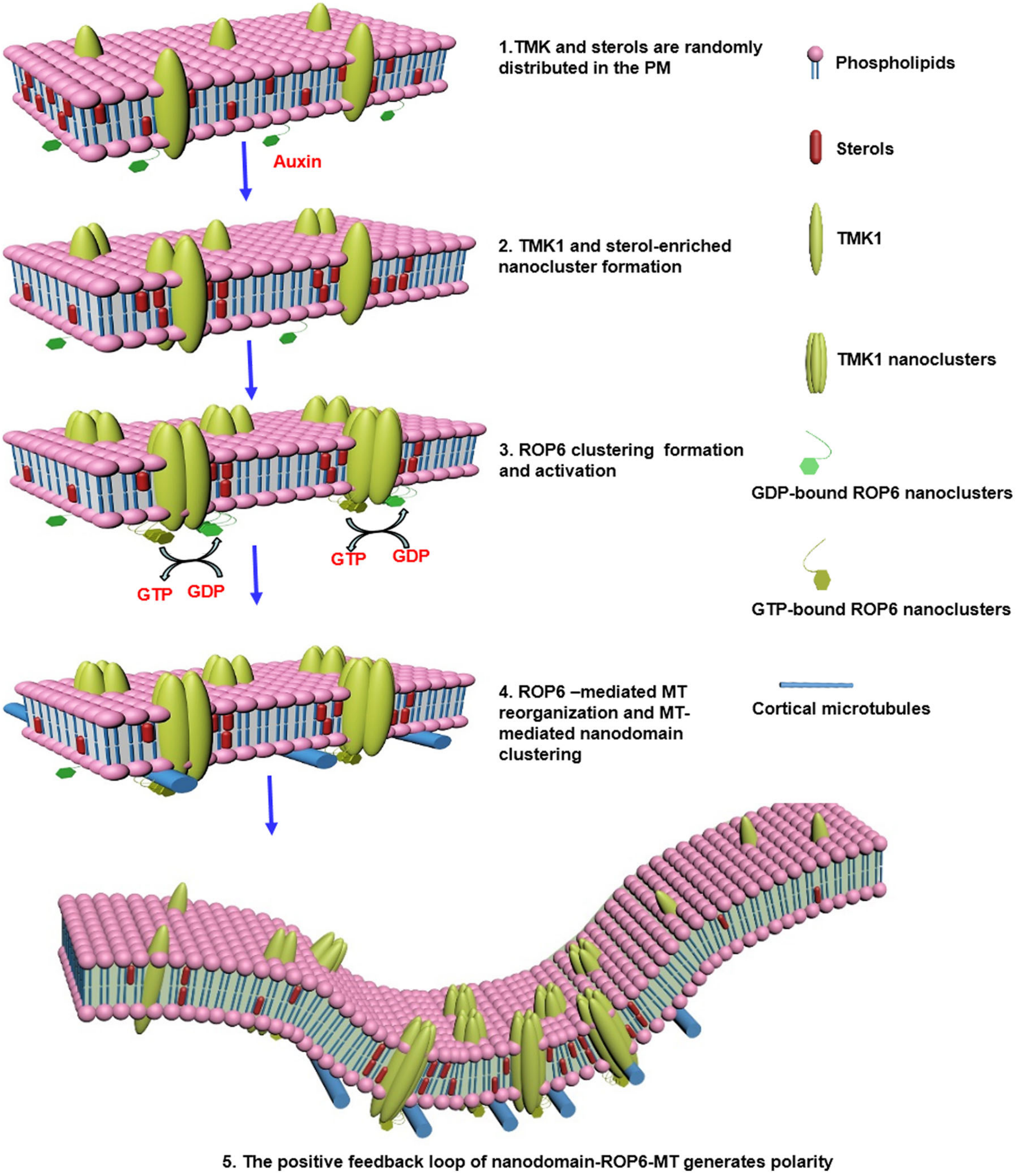

Figure 6. Working model for auxin-mediated multi-polarity establishment
Under normal conditions, the TMK receptor and sterols are randomly distributed in the plasma membrane (PM). The TMK proteins are first induced by auxin to form large nanoclusters. The enlarged nanoclusters lead to the formation of sterol-rich ordered lipid nanodomains that promote the formation and activation of ROP6 nanoclusters. Active ROP6 interacts with downstream effectors and promotes the cortical microtubule (CMT) ordering, which in turn promotes larger TMK1 nanoclusters and lipid nanodomains. The positive feedback loop between lipid nanodomain-ROP6 signaling and CMT ordering eventually leads to the establishment cell polarity.

sterols at the plasma membrane, which in turn promotes its downstream effector ROP6 (Xu et al. 2014) to cluster together and be activated (Figure 6). The activation of ROP6 signaling in the lipid rafts cross talks with the cortical microtubule, so that the protein nanoclusters are stabilized (Xue et al. 2019).

Besides sterol, phospholipid signaling also has a role in regulating plant cell polarity. Phospholipids may contribute to the establishment and maintenance of cell polarity by providing either local docking places or substrates for the local generation of secondary signaling molecules (Fischer et al. 2004). The *Arabidopsis thaliana* serine/threonine kinase D6 PROTEIN KINASE (D6PK) colocalizes with PINs and activates PIN-mediated auxin efflux in regulating plant development (Zourelidou et al. 2009; Willige et al. 2013; Barbosa et al. 2014). The association of D6PK with PINs is dependent on the phospholipid composition of the plasma membrane, as well as the phosphatidylinositol phosphate 5-kinases PIP5K1 and PIP5K2 in epidermal cells of the primary root (Stanislas et al. 2015). D6PK binds the poly-acidic phospholipids through a poly-basic lysine-rich motif that is required for proper PIN3 phosphorylation (Barbosa et al. 2016).

Protein phosphorylation

Protein phosphorylation catalyzed by kinase enzymes is a posttranslational modification that occurs on serine, threonine, or tyrosine residues. The reverse process of phosphate groups removal is mediated by protein phosphatases. In addition to many other regulations, the phosphorylation status of proteins also serves as an intrinsic cue for protein polar delivery, including both membrane-embedded and membrane-associated proteins in plants.

The PIN protein polarization is regulated by the PINOID (PID) kinase in *Arabidopsis* (Friml et al. 2004; Huang et al. 2010). An antagonistic function of the serine/threonine PID kinase and protein phosphatase 2A (PP2A) in the polar PIN trafficking was demonstrated by a genetic study of *pp2a* and *pid* mutants in embryo and root development (Michniewicz et al. 2007). An evolutionarily conserved phosphorylation site within the central hydrophilic loop of PIN proteins is important for the apical and basal polar PIN localizations, consequently, the redirection of auxin fluxes between the cells (Dhonukshe et al. 2010; Huang et al. 2010; Zhang et al. 2010). In addition, the polar localization of the transmembrane transporter NIP1;5 is also dependent on phosphorylation in the TPG repeat that is necessary for the efficient transport of boron in roots (Wang et al. 2017a).

Protein phosphorylation is also influential for peripheral membrane proteins to polarize. One prominent example is that the MAPK scaffold protein BASL needs to be phosphorylated to translocate from the nucleus to the PM polarity site (Zhang et al. 2015). On the other hand, the BIN2 kinase also phosphorylates BASL and POLAR to regulate their subcellular localization (Houbaert et al. 2018), but the molecular machinery and mechanisms by which BASL and POLAR are polarized remain unknown.

Others

Plant cells have a complex extracellular matrix, the cell wall, which seems to provide a plant-specific mechanism for cell polarity maintenance (Feraru et al. 2011). The PIN polarization in plant cells is maintained by the connections between the polar domains at the PM and the cell wall (Feraru et al. 2011).

The plant hormones auxin and cytokinin mutually coordinate their activities to control various aspects of development (Vanstraelen and Benkova 2012). Cytokinin modulates endocytic trafficking of PIN1 to control plant organogenesis (Marhavy et al. 2011). Specifically, cytokinin enhances the PIN1 depletion at specific polar domains, thus rearranging the cellular PIN polarities directly regulating the auxin flow direction. Cytokinin signaling also regulates pavement cell morphogenesis in *Arabidopsis*, likely acting through the ROP signaling (Li et al. 2013).

Calcium, a second messenger, is an organizer of cell polarity in plants. Calcium signaling plays multiple roles by (i) providing a hallmark for cell polarity; (ii) coordinating differential membrane trafficking; (iii) controlling cytoskeleton dynamics; (iv) interconnecting with ROP polarization; (v) integrating mechanical polarity signals (see review in Himschoot et al. 2015). It has been well known that the tip-focused [Ca^{2+}] gradient is an important factor for localizing growth of the elongating root hair and pollen tube to the apex (Bibikova et al. 1997). More recently, it was found that calcium signals are necessary to establish auxin transporter polarity in plant stem cell niche (Li et al. 2019).

CONCLUSION

During the past years, research has been mainly focused on revealing the regulatory mechanisms for the transmembrane proteins to be polarized, such as the PIN auxin effluxers and the boron transporters. Despite the knowledge obtained to explain ROP polarization, there was almost nothing known about how membrane peripheral proteins are polarized in plant cells. Many outstanding questions remain in the field of plant cell polarity to address how protein polarization is initiated, maintained, and regulated in plants. For example, what are the orientation cues for cell polarity or protein/lipid polarization in plant cells? With regards to PIN polarization, auxin itself with its local source directs its transportation and the orientation of developmental axis in *Arabidopsis* (Robert et al. 2013). The finding that auxin induces nanoclustering of the PM proteins was exciting (Xue et al. 2019), but the identity of the cell surface receptor remains to be established. With regard to the polarization of membrane associated proteins, such as D6PK, BASL, and POLAR, the identification of their binding partners (proteins or lipids) will likely provide insights suggesting how they can be possibly delivered and maintained at the polarity site. Also, it was noted that the TGN as a membrane sorting platform in plant cells plays critical roles in the establishment of the PM polarity domains. Among the few key regulators discussed earlier for their roles in establishment PIN polarization, the ARF GEF GNOM appeared to control both auxin transport and auxin signaling to establish tissue polarity (Verna et al. 2019). How GNOM functions at the Golgi/TGN to instruct PM polarity is a major mystery. In the future, more studies are desired to identify new regulators, which are dependent or independent of the membrane systems, towards understanding the molecular mechanisms by which cell polarity is initiated and maintained in plant cells.

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